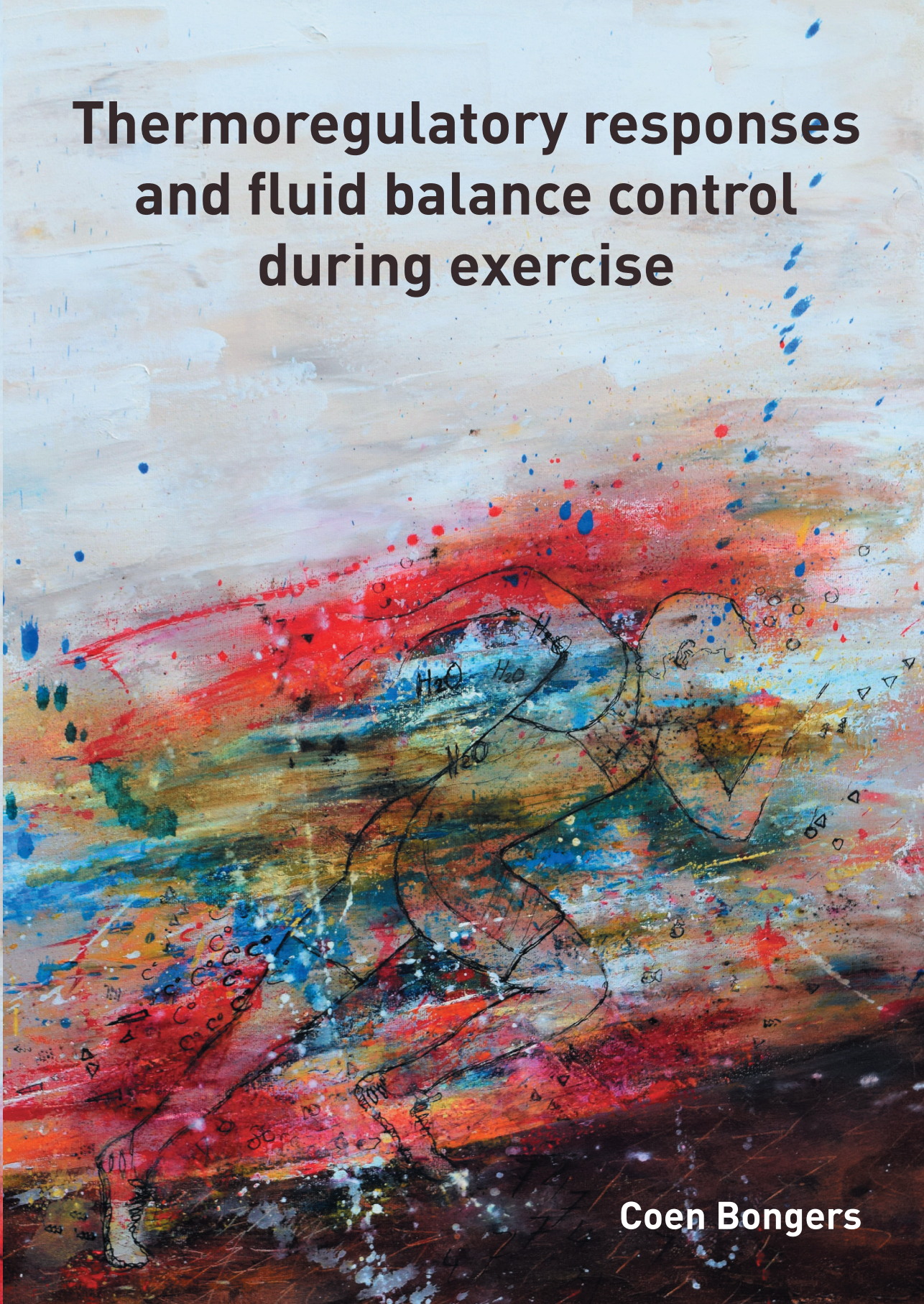
Thermoregulatory responses and fluid balance control during exercise



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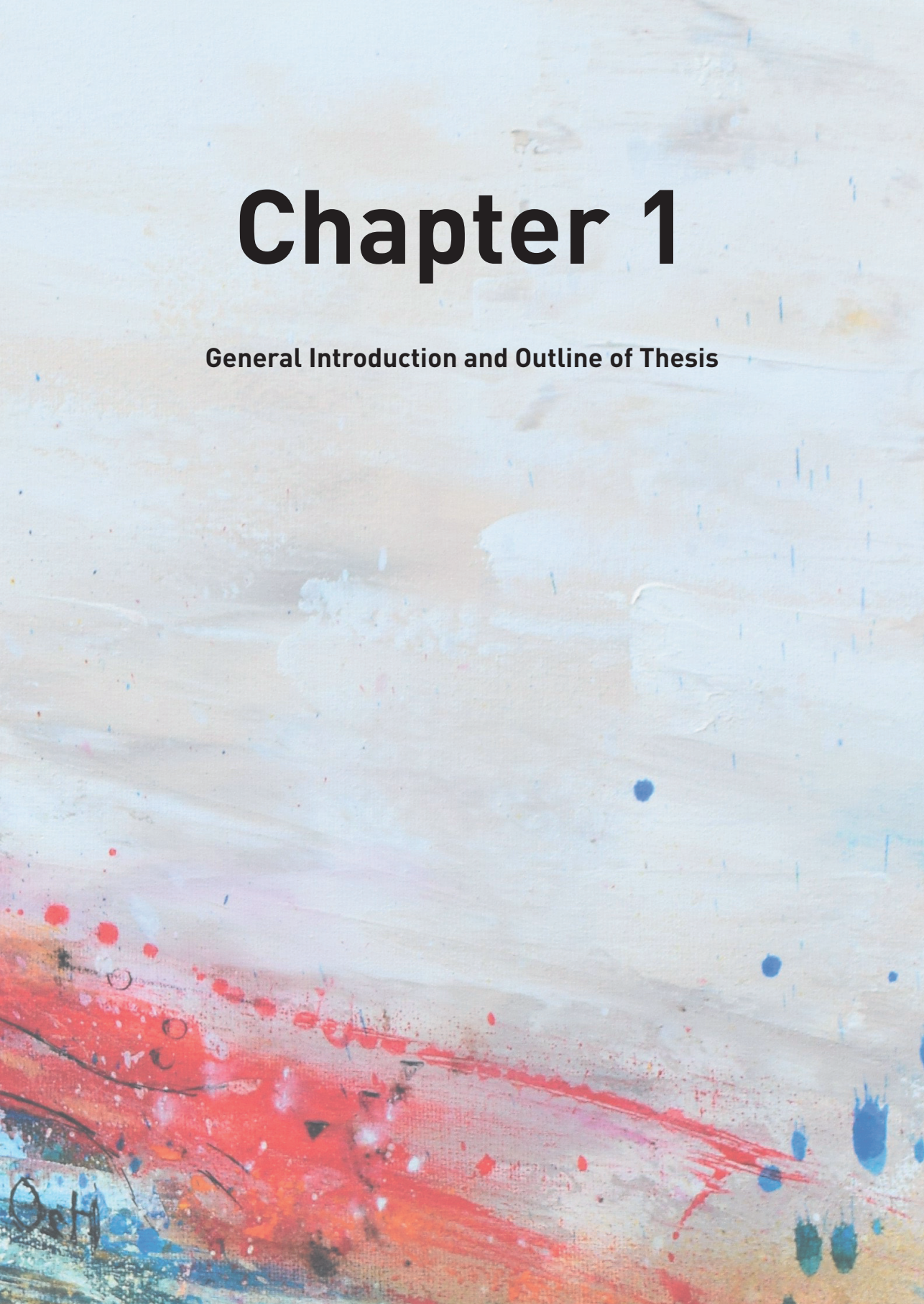
General Introduction and Outline of Thesis



In ancient times body heat was already considered as a vital sign. According to Hippocrates (460-377 before Common Era (BCE)), "fever originated with excess of bile, which was consistent for many infections of that era. Wherever bile is heated, all the rest of the body is heated along with it, and this is called fever"[1, 2]. Thereafter, Aulus Celsus (25 BCE – 50 Common Era (CE)) emphasized the value of body temperature and pulse rates, obtained using direct cutaneous palpation, to measure fever[3]. Claudius Galenus (128-198 CE) further elaborated on this theory and stated that an increased body temperature is the primary characteristic of fever, which emphasized the importance of body temperature palpation[3, 4].

However, it lasts until the 16th century until Galileo Galilei (1564-1642) invented the first instrument to measure changes in temperature, which was based on the expansion of water with increased temperature[2, 5, 6]. However, the thermometer of Galileo did not contain a thermal scale. In 1612, Sanctorius Sanctorius (1561-1631) was the first to put a numerical scale on his thermometer, in which de temperature of snow and the temperature of a candle flame were used as reference values[2, 6]. In 1714, Daniel Gabriel Fahrenheit (1686-1736) was the first to develop a mercury based thermometer, as mercury expanded and contracted more rapidly than water and other mixtures[3, 7]. Thermometric experiments in the 18th century resulted in improvements to reliable quantitative temperature scales. In 1724, Fahrenheit introduced his standard temperature scale (°F). The Fahrenheit scale defined 32°F as the freezing point of water and 212°F as the boiling point of water[2, 3]. Furthermore, he defined the normal body temperature to 100°F. Anders Celsius (1701-1744) introduced his temperature scale in 1742, in which the freezing and boiling point of water were defined as 100°C and 0°C, respectively[2, 3]. The Celsius scale was reversed later by Carolus Linnaeus (1707-1778).

Sanctorius was in 1612 also the first to use the thermometer in clinical settings, in which a patient's body temperature was estimated using expired air from the mouth[2, 5]. However, the thermometer was not in general use until Hermann Boerhaave (1668-1738), Gerard van Swieten (1700-1772) and Anthonie de Haen (1704-1776) started to implement the thermometer in clinical routine in the 18th century[3, 7]. The interest in normal body temperature gained attention in the late 18th and 19th century. As a result, Carl Wunderlich (1815-1877) published his results regarding the course of temperature in diseases in 1868[8]. Moreover, Wunderlich defined 37°C as the normal body temperature and regarded 38°C as the upper limit of normal temperature, with exceeding levels defined as fever[8].

1. Physiology of the Human Thermoregulation

When we discuss the temperature of our body, we refer to the central core body temperature (Tc) and the peripheral shell. The central Tc reflects the temperature of the brain, and central thoracic and abdominal area, whereas the peripheral shell temperature consists of the temperature of the skin, subcutaneous tissue and muscles[9]. Both, the central and peripheral

temperature are continuously monitored by afferent sensors and neuronal feedback is given to the thermoregulatory center (Figure 1)[10, 11]. The thermoregulatory center is located in the preoptic area of the anterior hypothalamus in the brain[12-14]. This thermoregulatory center consists of heat-sensitive neurons (~20%), cold-sensitive neurons (~10%) and thermoinsensitive neurons (~70%)[12, 13]. The thermosensitive neurons continuously monitor the temperature of the brain blood flow and therefore detect changes in Tc[12]. The afferent neuronal feedback from the central and peripheral thermoreceptors is continuously compared with a critical threshold temperature, which is often defined as the set-point temperature[11, 13]. Subsequently, any difference between the afferent temperature information and the set-point temperature induces a thermoregulatory response to either stimulate heat production or heat dissipation, returning the Tc back to the set-point temperature. These responses can include non-shivering thermogenesis (heat production by brown fat or metabolic processes), shivering, cutaneous vasoconstriction and vasodilation, evaporation and behavioral responses[12, 15].

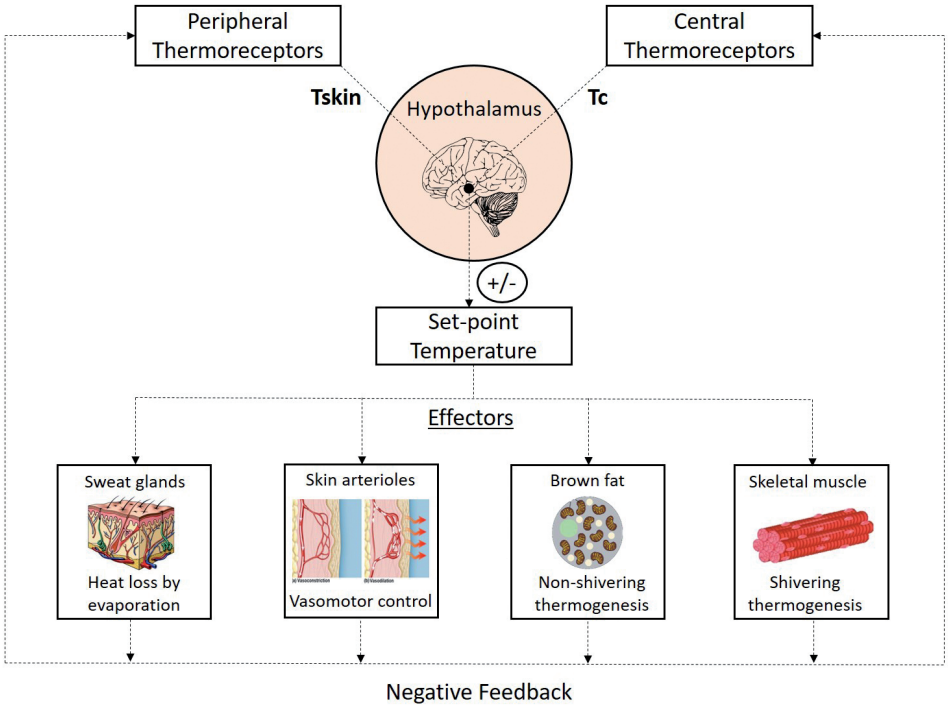


Figure 1. Schematic overview of human thermoregulatory system. Tskin = skin temperature and Tc = core temperature

Set-point temperature

The continuous interaction between the thermoregulatory center and the central and peripheral feedback neurons contribute to the fact that we can regulate our Tc within a narrow range, which is typically between 36.2°C and 37.7°C[8, 16-18]. As a result of the circadian rhythm, the set-point temperature varies throughout the day (±0.5°C), with the minimum Tc in the morning (05:00-06:00 hour) and maximum Tc in the evening (21:00-22:00 hour)[19-21]. The circadian changes in Tc can be explained by a rhythmic input from the suprachiasmatic nuclei on the thermoregulatory center, resulting in a modulation of the set-point temperature and an alteration in thresholds for vasodilation and sweating[22]. The set-point temperature is also influenced by age[21], since elderly have a lower resting Tc, a reduced vasodilatory capacity and a decreased sensitivity of the thermal receptors[23, 24]. Similarly, the menstrual cycle influences set-point temperature[25]. The Tc in men and women are comparable when women are in the follicular phase, whereas the Tc is ~0.4°C higher in women in the luteal phase compared to the follicular phase[25, 26].

Thermoregulation in rest

In order to maintain a relatively constant Tc, a fine balance between heat production and heat loss must be maintained[27, 28]. The maintenance of Tc around the set-point temperature is accomplished by a number of different physiological responses. Heat production derived from the cellular metabolism at rest, which is defined as basal metabolic rate, is the primary source of heat gain at rest[28, 29]. Heat loss, which can be defined as heat transfer between the body and an external environment, occurs through I) heat convection, heat transfer to or from the body to surrounding moving fluid or air, II) heat radiation, heat transfer to or from a body via radiation from higher to lower energy surfaces, III) heat conduction, heat transfer from warmer to cooler subjects through direct physical contact, and IV) heat evaporation, heat loss due to heat vaporization of sweat from the skin surface[27, 28, 30]. Heat evaporation is the most effective route of heat loss, and it is the primary heat loss mechanism during heat exposure or during exercise[27, 28, 31].

In normal circumstances our Tc is higher than the surrounding ambient temperature. Since heat transfer always occurs down a gradient (from hot to cold), we continuously lose heat to our environment via radiation, conduction and convection[27]. As humans are endothermic homeotherms, we are able to produce our own body heat via our metabolism[27] and therefore maintain thermal homeostasis. The thermal balance is an equilibrium between heat production and heat loss, which can be disturbed for example by physical activity[10].

Thermoregulatory responses during exercise

Exercise represents a major challenge to whole-body homeostasis and provokes widespread changes in numerous cells, tissues and organs that are caused by or are a response to the

increased metabolic activity of contracting skeletal muscle, which is associated with an increased muscle energy and oxygen demand[32]. However, during exercise only ~20–30% of the produced energy is converted to mechanical work, whereas ~70–80% of the energy is released as heat[33, 34]. The increased metabolic heat production during exercise, in combination with impaired heat loss possibilities by an elevated ambient temperature or humidity, creates a major physiological challenge for the body[31]. As a consequence, the heat production during exercise typically exceeds the body's capacity to lose heat, resulting in an increased Tc[10, 35]. Any increase in Tc above its normal range (set-point) is defined as hyperthermia[30, 36], while a further increase in Tc (>40°C, exertional hyperthermia) can even lead to heat-related illnesses (i.e. heat exhaustion and heat stroke) or even death[27, 36, 37].

Methods to measure Tc

In order to protect individuals from severe heat-related illnesses, it is important to monitor Tc and anticipate on high Tc levels. Unfortunately, accurate measurement of Tc is difficult. Intra-pulmonary arterial temperature is generally considered as gold standard for Tc[38], but this method is invasive and only applicable in clinical settings. Others methods to monitor changes in Tc are based on temperature recordings of the external tympanic membrane, mouth, rectum and esophagus[39, 40]. These measurement sites are not optimal to measure Tc, given their invasive character, slow response time, methodological difficulties and/or the potential bias by environmental conditions[39-41]. Alternatively, athletes have been using ingestible temperature capsules to wirelessly measure gastro-intestinal temperature as a valid, reliable and easily applicable surrogate marker of Tc[39, 42, 43]. Furthermore, the use of ingestible temperature capsules is suitable for field-based conditions, which is of great importance since exercise-induced elevations in Tc are generally higher in field compared to laboratory-based settings[44]. Further insight into the measurement of the gastro-intestinal temperature using ingestible temperature capsules is therefore of great importance.

2. Physiology of the Human Fluid balance

Water is the most abundant compound in the body and is an essential regulator of the internal environment[45]. Moreover, water plays a key role in the digestion, absorption, transportation, and use of nutrients, is necessary for energy production, has a crucial role in the safe elimination of toxins and waste products, and is also essential for the thermoregulation[46]. In fact, water deprivation will lead to death within a few days, especially in a hot environment[47]. Therefore, it is important that fluid intake is sufficient to compensate for fluid loss.

The fluid balance, normally indicated by total body water, is a continuous interaction between fluid intake (fluid gain) and fluid loss. Fluid gain occurs primarily through food and drink intake, but also by metabolic water formation (Figure 2)[48, 49]. Fluid loss mainly occurs by renal water clearance, which is the loss of water through the process of filtration in the kidneys that result

in the output of urine[48]. Furthermore, fluid loss occurs due to respiratory water loss (the loss of water associated with the respiration), gastrointestinal water loss (feces) and cutaneous water loss (sweat)[48, 49]. A normal total body water content is often defined as euhydration[50], in which the body mass is usually used to represent changes of body water[51, 52]. Euhydration is not an individualized specific body mass, but a body mass that fluctuates within a narrow range (~0.7%) for repeated days[53]. A reduced total body water below the average baseline value is defined as dehydration. Although no consensus exists regarding the exact cut-off value for dehydration[50, 54], it is often described as a body mass loss ≥2%[55, 56]. Hyperhydration (or overhydration) refers to the state in which the fluid intake temporarily increases total body water above baseline values prior to its removal of the kidneys[50]. As a consequence of a less effective compensatory mechanism (urine production) during exercise, the overconsumption of fluids may result in hyponatremia[55].

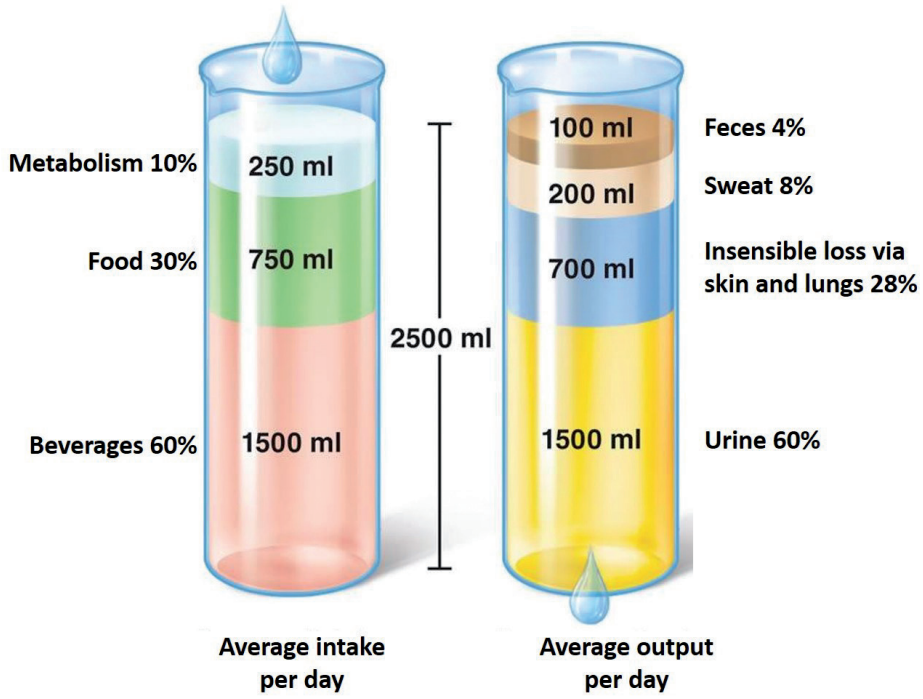


Figure 2. Overview of the average daily fluid intake and output

Body fluid compartments

Mean values for total body water volume are approximately 60% of body mass (0.6 L/kg^{-1} body mass)[50, 57, 58], with a range from 45% to 75% of body mass[48, 58]. The total body water differs

between sexes and varies across the lifespan, with lower values for females and at older ages[45, 57]. Furthermore, differences in body composition have impact on total body water[59, 60]. The total body water can be divided into an intracellular and an extracellular compartment[51, 57, 61]. The intracellular compartment consists of the fluid within tissue cells, comprising of ~0.4 L/kg^{-1} body mass. The extracellular compartment on the other hand is all the fluid outside of cells, which comprises ~0.2 L/kg^{-1} body mass. Moreover, 75% of the extracellular fluid is distributed interstitially (~0.15 L/kg^{-1} body mass) and 25% intravascularly (plasma volume, ~0.05 L/kg^{-1} body mass)[51, 57, 61]. In a normal man (70 kg) the body consists of ~42 L of water; ~28 L in the intracellular compartment and ~14 L in the extracellular compartment (~10.5 L interstitial compartment and ~3.5 L intravascular compartment, Figure 3). It is importantly to note that these are not static volumes, but these represent the net effect of dynamic fluid exchange with varying turnover rates between compartments[62]. Perturbations in fluid balance such as exercise or heat exposure will greatly modify the net volumes and turnover rates between fluid compartments[63]. Moreover, the continuous exchange of fluid between compartments, driven by osmotic-oncotic gradients and hydrostatic pressure, promotes the maintenance of the fluid balance homeostasis[64].

Fluid balance regulation at rest and during exercise

At rest, our body is able to regulate fluid balance (fluid intake = fluid loss) and to maintain a sufficient total body water, which is mainly attributable to the intrinsic thirst sensation. Thirst is stimulated or inhibited by the neuro-endocrine and renal system in response to changes in total body water and plasma osmolality[60]. As a result, small changes in fluid balance, resulting in dehydration or hyperhydration, can be restored[60]. However, the fluid balance can be disturbed very easily during exercise or after excessive fluid intake. This might result in a water deficiency or a water surplus. The exercise-induced increase in Tc stimulates cutaneous water loss due to its cooling effect through evaporation. The increased sweat production leads to fluid loss derived from all the body compartments, including the intravascular compartment[63, 65]. Moreover, the precursor fluid for sweat is interstitial fluid[63]. Excessive fluid loss through an increased sweat secretion will therefore result in a relatively large reduction in both interstitial and intravascular volume. The drop in intravascular volume may cause a fall in cardiac output and arterial blood pressure. Therefore, a redistribution of body water occurs to limit intravascular volume loss and ensure circulatory stability[63].

When fluid loss exceeds fluid intake it causes dehydration, which results in an increased plasma osmolality and a decreased circulating blood volume. The increased osmolality will be noticed by osmoreceptors located within specific regions of the hypothalamus, the organum vasculosum lamina terminalis and the subfornical organ[66]. The osmoreceptors stimulate the thirst sensation and the secretion of the antidiuretic hormone vasopressin[67-69]. The decreased circulatory blood volume on the other hand is sensed by baroreceptors, which regulate the

release of vasopressin as well[70]. The increased thirst sensation stimulates fluid intake, and therefore the restoration of circulating volume and osmolality[68]. The release of vasopressin activates aquaporin-2 water channels, which enables the reabsorption of water in the loop of Henle in the kidneys[67, 70, 71]. This protects the body for a further decrease in circulating volume. Furthermore, the renin-angiotensin-aldosterone system (RAAS) will be activated when the circulating blood volume decreases[72]. The aldosterone hormone regulates the sodium reabsorption and concomitant fluid reabsorption by the kidneys, in order to restore body fluid homeostasis[72]. In case of hyperhydration the opposite occurs: a decreased secretion of vasopressin and an elevated urine excretion, which restores fluid balance[73].

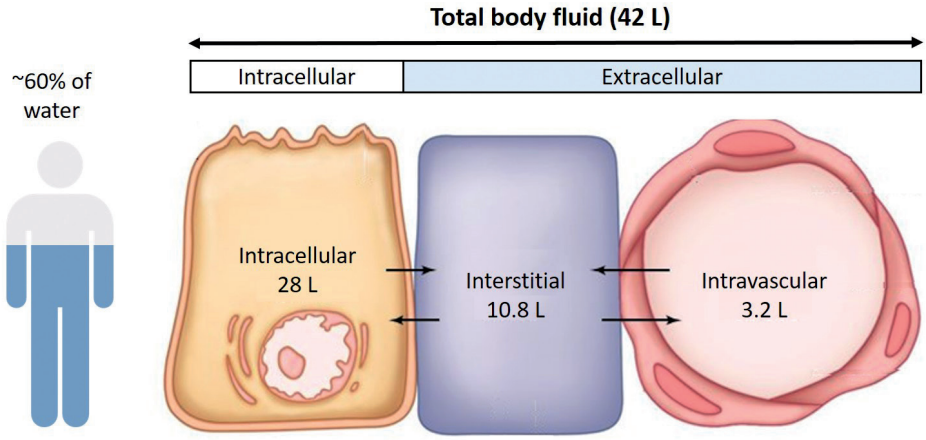


Figure 3. The distribution of total body water for an average 70 kg male, which consists for ~60% of water. The fluid is divided into an intracellular, interstitial and intravascular compartment. The dynamic fluid exchange between compartments, driven by osmotic-oncotic gradients and hydrostatic pressure, supports fluid homeostasis.

Role of the kidneys

Under normal circumstances the kidneys are the primary controllers of water balance. In fact, the most important function of the kidneys is the homeostatic regulation of the fluid and electrolyte balance[74]. The kidneys maintain normal blood concentrations of water and electrolytes by balancing intake of those substances with their urinary excretion. For that purpose, the kidney is the most perfused organ in the human body, receiving 20% to 25% of the cardiac output[75, 76]. During exercise a redistribution of blood to the active body parts occurs, which results in an absolute decrease in renal blood flow[76, 77]. As a result, the renal filtration decreases and the glomerular permeability increases, which may induce proteinuria[78-80]. Additionally, the increased fluid loss due to elevated sweat rates and associated occurrence

of dehydration stimulate the kidneys to preserve and reabsorb water by upregulating the vasopressin (AVP) secretion and activating renin-angiotensin-aldosterone system (RAAS)[70, 72]. Exercise is a stressful condition for the kidneys that may impact on kidney function and the development of 'temporary' kidney damage. From previous studies it is known that strenuous exercise could result in a decreased kidney function[78, 81], which is typically restored within 24 hours[81]. Other studies found that a bout of short-term high intensity exercise and completing a (ultra) marathon increased biomarkers for kidney injury[82-84]. However, it is unknown whether the kidney's response to repetitive exercise is similar to that of a single bout of prolonged exercise. Since training programs, especially for endurance athletes, consist of exercise bouts on consecutive days, it is relevant to know whether renal function might be affected in a cumulative way. Furthermore, it is unknown whether the kidney's response to acute exercise is similar to that of prolonged exercise.

3. Effects of hyperthermia and dehydration on exercise performance

It is widely accepted that hyperthermia increases the physiological strain on the body and can impair exercise capacity[85-87]. It has been shown that cycling efficiency drop by about 1% for every degree increase in CBT[88]. Moreover, there is evidence of exercise-induced fatigue beyond a Tc threshold of >40°C[86], and a Tc >40.5°C may lead to the development of heat-related illnesses[37]. Hyperthermia-induced fatigue primarily relates to changes in the central nervous system that lead to central fatigue and impairments in cardiovascular function[89]. As a consequence, the arterial oxygen delivery will decrease and the aerobic energy turnover within the exercising muscles deteriorates, resulting in a performance decrement[90, 91]. It is hypothesized that the reduction in work rate regulated by the central nervous system may be a neural safeguard mechanism to terminate exercise once a critically high Tc is obtained[87, 92]. An alternative hypothesis suggests that the rate of heat gain is continuously detected by our body, which could anticipatorily adjust the work rate to ensure that the exercise task can be completed within the homeostatic limits of the body[93, 94]. During prolonged exercise in the heat, the exercise intensity decreases with higher Tc, resulting in an impaired exercise performance. Previous studies showed significant increases in Tc in athletes exercising in cold[95], hot[96] and humid[97] environmental conditions. However, in hot and humid environmental conditions the possibilities to lose heat by evaporation and radiation are limited, resulting in performance decrements and a higher risk to develop heat-related illnesses[11].

Next to the negative impact of a high Tc, disturbances in fluid balance and associated dehydration may also have a detrimental effect on exercise performance[98]. In short, dehydration decreases the intravascular volume (plasma volume) and increases the plasma osmolality in proportion to the decrease in total body water[59]. The increased plasma osmolality can delay thermoregulatory cutaneous vasodilation and sweating by elevating the threshold for both[99, 100]. Thus, dehydration reduces the sweating rate, decreases evaporative heat loss[101]

and increases heat storage[99, 102]. Moreover, it is suggested that every 1% dehydration leads to an extra increase in Tc of ~0.15°C[101, 103, 104]. In addition to the negative impact on performance, dehydration may also increase the risk on developing heat-related illnesses[105].

Second, as a result of a reduced circulating plasma volume, heart rate increases due to the decrease in stroke volume[106]. The increase in heart rate can initially preserve cardiac output, however the heart rate fails to compensate cardiac output when dehydration progresses. As a consequence of the higher heart rate and lower cardiac output, the rate of perceived exertion increases and exercise performance decreases[107]. The combination of heat strain and dehydration during exercise causes competition between the central (maintaining central circulation and cardiac output) and peripheral circulation (cutaneous vasodilation to stimulate heat loss) for the limited blood volume[98], which further increases the physiological strain for a given exercise[101, 108]. The overall consensus in literature is that dehydration of ≥2% body mass loss represents a threshold at which endurance exercise performance in the heat becomes impaired[56, 98, 109]. Interestingly, a recent meta-analysis suggested that the 2% body mass rule does apply at fixed exercise-intensity in lab-based conditions, but not to athletes performing in an outdoor exercise event[110]. In field-based conditions dehydration up to 4% of body mass does not degrade exercise performance[110, 111]. Although the dehydration threshold is not exactly defined, rehydration strategies during exercise should focus on avoiding body mass losses of 2% or more.

4. Cooling Interventions

Interventions to counteract the negative effects of Tc elevations on exercise performance and the development of heat-related illnesses are of great importance. Cooling the body prior to (pre-cooling) or during exercise (per-cooling) has proven to be effective in decreasing Tc and improving exercise performance[112]. The basis of pre-cooling and per-cooling strategies is to reduce heat stress of the thermoregulatory system[112, 113]. Pre-cooling can be described as the rapid removal of heat from the body before exercise to create a larger heat storage capacity[114]. The beneficial effects of pre-cooling normally attenuate after 20-25 minutes of exercise[115]. Therefore, the use of cooling strategies during exercise (per-cooling) became of greater interest. The use of per-cooling may elongate the duration of the beneficial effects of the cooling intervention. Furthermore, the level of thermal strain during exercise is higher compared to resting conditions[35], which suggest that per-cooling has a large potential to prevent significant thermal strain and maintain exercise performance. Additionally, the use of cooling strategies after exercise (post-cooling) can reduce the Tc directly after exercise, to enhance recovery from exercise and to reduce the exercise-induced muscle soreness[116].

Many pre-, per- and post-cooling techniques were proven to be effective, ranging from whole body cooling such as cold water immersion[117], cold air exposure[118], and cryotherapy[119] to

local cooling using cooling vests[120, 121], facial wind or water spray[122] or cooling packs[123, 124]. The use of internal cooling strategies such as the ingesting of cold water or ice slurry are also effective in improving exercise performance[117, 125]. Furthermore, a combination of cooling techniques is often used to obtain a greater cooling power and larger reduction in Tc[123, 126]. The beneficial effects of cooling may vary between cooling techniques and timing of cooling. Better insight into the mechanisms and potential benefits of cooling techniques is therefore necessary to identify 'the best practice' to improve exercise comfort and exercise performance.

5. Outline of Thesis

It is well known that exercise leads to an increase in Tc and to disturbances in fluid balance, which can even result in heat-related illnesses and/or severe dehydration. However, the thermoregulatory burden during exercise may increase even further with global warming[127, 128]. Nowadays this is particularly relevant for professional athletes, since future major sport events will be more often organized in hot and humid ambient conditions (i.e. Athletic World Championships of Doha 2019, Olympic Games of Tokyo 2020, FIFA World Cup in Qatar 2022). A better understanding of the thermoregulatory and fluid balance responses to exercise is therefore of great importance, as well as a better insight into interventions to counteract the negative consequences of Tc elevations on exercise performance. Therefore, the general aim of this thesis was to evaluate the thermoregulatory and fluid balance responses to exercise in young and elderly individuals, using State-of-the-Art equipment. Second, we aimed to get more insight into cooling strategies to determine the most beneficial cooling strategy to improve exercise performance in the heat.

Chapter 2-4. An accurate measurement of Tc is of great importance to measure thermoregulatory strain at rest and during exercise. Therefore, we described in **Chapter 2** the use of an ingestible telemetric temperature capsule to measure the intestinal temperature as a surrogate marker for Tc. In **Chapter 3** we assessed the validity and reliability of a new ingestible telemetric temperature capsule system using an ex-vivo water bath, in which the water temperature was increased step wisely from 33°C to 43°C. Subsequently, in **Chapter 4** we compared the validity, reliability and inertia characteristics of four different temperature capsule systems, including the new myTemp system, using an ex-vivo water bath.

Chapter 5-6. The negative impact of a high Tc on exercise performance is already described in previous studies[11, 86]. Interventions to attenuate the increase in Tc could therefore be effective in improving exercise performance in the heat. The use of cooling strategies prior to, during or directly after exercise are proven to be suitable to decrease the Tc[112]. In **Chapter 5** we performed a meta-analytical review to examine the effects of cooling prior to or during exercise on exercise performance. Furthermore, we determined the most beneficial timing of cooling

and cooling strategy. In **Chapter 6** the potential mechanisms for the beneficial effects of cooling on exercise performance were described.

Chapter 7. The use of cooling during exercise may elongate the effectiveness of the intervention to limit the increase in Tc and therefore enhance exercise performance. Furthermore, using a light-weight cooling vest, which covers a large part of the body, is easily applicable during exercise in lab- and field-based settings and may result in a further increase in exercise performance levels. Therefore, we examined in **Chapter 7** the effects of wearing a cooling vest during a 5 km running time trial on exercise performance in moderate ambient conditions.

Chapter 8. Individuals with a spinal cord injury are known to have a reduced afferent input to the thermoregulatory center[129] and an impairment of the efferent system leading to an attenuated sweating response and vasomotor control below the level of the lesion[130, 131]. Spinal cord injured individuals are therefore at greater risk to develop heat-related illnesses. Therefore, the aim of **Chapter 8** was to examine the effects of wearing a cooling vest during exercise on Tc response of individuals with a thoracic SCI.

Chapter 9. Advanced age is associated with a negative impact on thermoregulatory and fluid balance responses during exercise[23]. Moreover, elderly have a decreased sensitivity of the thermal receptors, a less effective sweat response, a lower total body water and a decreased thirst sensation[23, 132, 133]. However, it is unknown whether thermoregulation and fluid balance control deteriorates further with aging or plateaus at some point. Therefore, in **Chapter 9** we assessed the differences in thermoregulatory and fluid balance responses to prolonged walking exercise in 60- versus 80-year-old participants of the Nijmegen Four Days Marches.

Chapter 10-11. Exercise and exercise-induced dehydration are known to alter kidney function and stimulate the kidneys to reabsorb water[79, 80]. These renal responses to exercise may lead to acute, but transient, kidney injury[83]. Athletes training programs usually consists of exercise bouts on consecutive days. Therefore, it is relevant to know whether renal function might be affected in a cumulative way. For that reason the purpose of **Chapter 10** was to examine the effect of a single versus repetitive bout of endurance exercise on markers for kidney injury. Furthermore, previous studies primarily focus on the effects of exercise-induced dehydration on kidney function and kidney injury. However, the acute effects of exercise, with less dehydration but with ischemic kidney stress, are not studied yet. Therefore, in **Chapter 11** we distinguish between the effects of acute and prolonged exercise on kidney function and kidney injury.

Chapter 12 provided a general discussion on the main findings of the present thesis. In this chapter we discussed the relationship between thermoregulation, fluid balance and exercise performance, in which we addressed the impact of cooling and aimed to identify the best practice cooling strategy to improve exercise performance. Furthermore, we speculated about the effects of global warming on thermal strain in healthy individuals and individuals with a compromised thermoregulation.

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Chapter 2

Using an Ingestible Telemetric Temperature Capsule to Assess Gastrointestinal Temperature During Exercise

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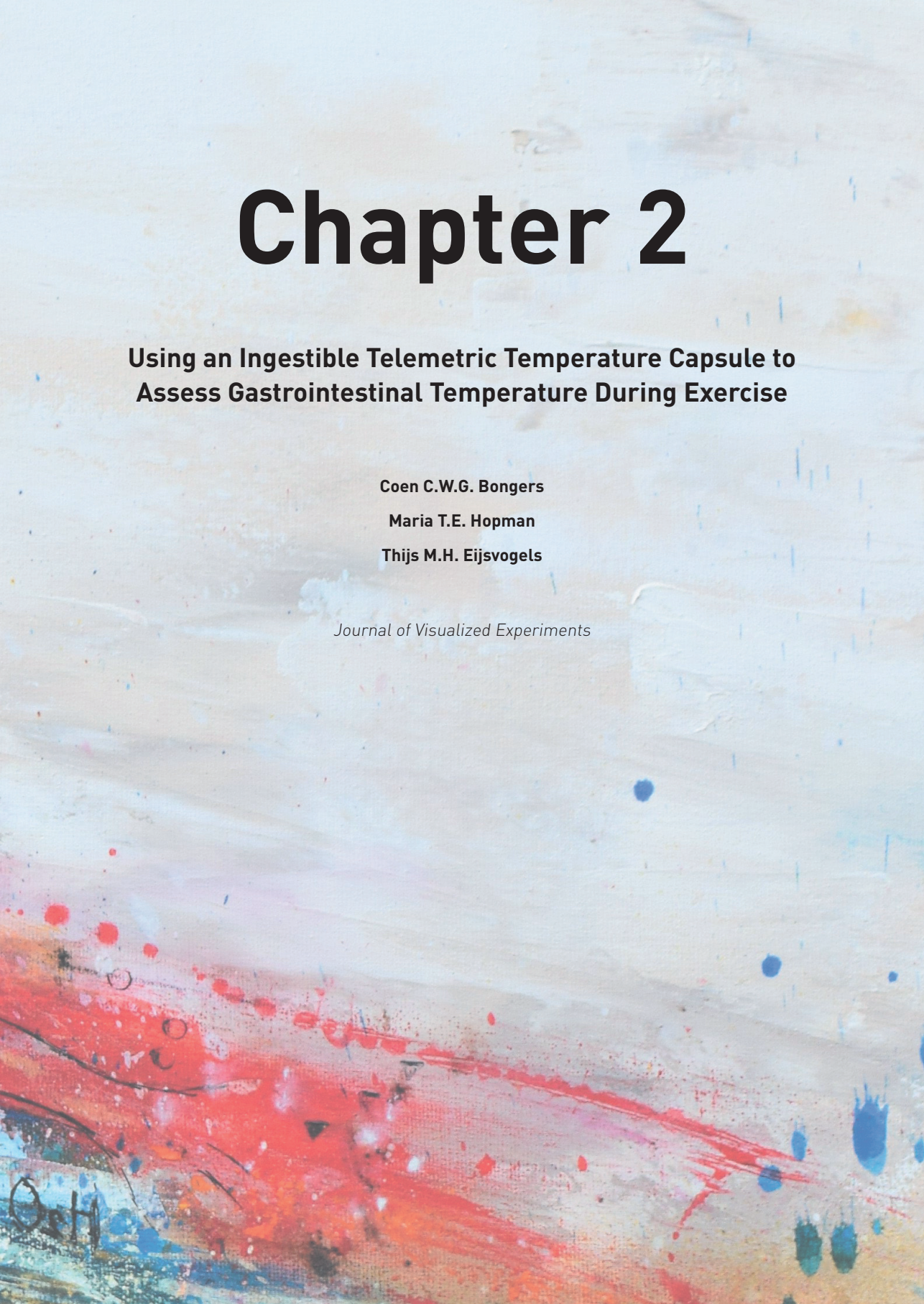
Journal of Visualized Experiments



Using an ingestible temperature capsule to assess gastrointestinal temperature during exercise

Applicable in field-based conditions

Rapidly monitors changes

Not biased by environmental conditions

In accordance with gold standard

Non-Invasive

Very accurate ±0.1°C

Considering that:

- Capsule ingestion ≥ 6 hours prior to the measurement
- Avoid interference between two temperature capsules
- Temperature capsule is relatively expensive (~$50)

Temperature capsules are valid and reliable to measure intestinal temperature in lab- and field-based conditions

ABSTRACT

Exercise results in an increase in core body temperature (Tc), which may reduce exercise performance and eventually can lead to the development of heat-related disorders. Therefore, accurate measurement of Tc during exercise is of great importance, especially in athletes who have to perform in challenging ambient conditions. In the current literature a number of methods have been described to measure the Tc (esophageal, external tympanic membrane, mouth or rectum). However, these methods are suboptimal to measure Tc during exercise since they are invasive, have a slow response or are influenced by environmental conditions. Studies described the use of an ingestible telemetric temperature capsule as a reliable and valid method to assess gastrointestinal temperature (Tgi), which is a representative measurement of Tc. Therefore, the goal of this study was to provide a detailed description of the measurement of Tgi using an ingestible telemetric temperature capsule. This study addresses important methodological factors that must be taken into account for an accurate measurement. It is recommended to read the instructions carefully in order to ensure that the ingestible telemetric temperature capsule is a reliable method to assess Tgi at rest and during exercise.

INTRODUCTION

The oxidation of substrates during muscle contractions, necessary to perform exercise and physical activity, importantly impacts our thermoregulatory system as only 20% is used for muscle power[1], whilst the majority of the energy is released as heat (80%)[2, 3]. As a consequence, the elevated metabolic heat production during physical activity and exercise typically exceeds the heat dissipation capacity[4, 5], resulting in an increase in core body temperature (Tc). Accordingly, Tc rises above the hypothalamic set point, which is defined as hyperthermia[6], and may even result in an attenuated exercise performance[5, 7, 8] and/or the development of heat-related disorders[4, 6]. For this reason it is important to accurately measure Tc during prolonged exercise and in particular in strenuous ambient conditions.

Literature describes that an ideal method to measure Tc should: 1) be easy applicable, 2) not be biased by environmental conditions, 3) have a high temporal resolution to rapidly monitor changes in Tc, and 4) have the capacity to detect small changes (Δ0.1°C) in core body temperature[9, 10]. An overview of the different methods to measure the Tc was given by the International Organization of Standardization (ISO 9886)[11]. It was stated that the esophageal temperature at the level of the left atrium provides the closest agreement with central blood temperature, while this measure is able to rapidly detect (minor) changes in temperature[12]. Although esophageal temperature measurements are generally accepted as the gold standard to record Tc, its invasive nature limits the practical use of this method. Alternative measures to monitor Tc rely on temperature recordings of external tympanic membrane, mouth, or rectum[12]. These measurement sites are not optimal to measure the Tc, given their invasive character, methodological difficulties and/or the potential bias by environmental conditions[9, 12-14] (Table 1). This highlights the need to explore alternative strategies to monitor (changes in) Tc.

Previous studies have described the use of an ingestible telemetric temperature capsule as an easily applicable, reliable and valid method to measure the Tgi, which is a representative estimation of Tc[9, 15]. Another, important, advantage of the temperature capsule is the suitability in field-based situations, which is of great importance since exercise-induced elevations in Tc are generally higher in field than in laboratory settings[23]. Currently, the temperature capsule is able to measure the Tgi every 10 seconds with an accuracy of ±0.1°C, which make this technique very suitable to measure the Tgi during an exercise event or an important match. Furthermore, in a study by Stevens *et al.*[24] is demonstrated that the telemetric temperature capsule may also be used to monitor gastrointestinal temperature. The ingestible temperature capsule is first described in 1961[25], and further developed at the Johns Hopkins University (Baltimore, USA) in collaboration with the Applied Physics Laboratory of the NASA. The result is a 20 x 10 mm capsule with a telemetry system, micro battery and a quartz crystal temperature sensor. The crystal sensor vibrates at a frequency relative to the temperature of

the surrounding substance. This temperature radio signal is transmitted through the body, which can be measured by an external recorder (Figure 1). Each temperature capsule has a unique serial and calibration number, which can be used by the recorder to covert the radio signal and measure the corresponding Tgi.

A small magnetic strip is attached to the outside of the temperature capsule, which deactivates the battery. When this magnetic strip is removed, the capsule is activated immediately and starts measuring Tc (Figure 2). Casa and colleagues[16] used six different techniques (gastrointestinal, rectal, aural, temporal, axial and forehead) to measure Tc, with the rectal temperature set as the reference value. They demonstrated that the gastrointestinal measurement of Tc with the temperature capsule is the only technique that shows good agreement with the reference Tc. Others investigated the relation between Tgi and rectal temperature and have shown a small but significant bias ranging from 0.07°C to 0.20°C[9, 15, 18, 22]. Although the direction and magnitude of the bias differed between studies, the Bland and Altman 95% limits of agreement were ±0.4°C, which is acceptable[9, 26]. Additionally, in a review by Byrne *et al.*[9] the Tgi is compared with the rectal and esophageal temperature (gold standard) as a measure for the Tc. They demonstrate that the Tgi measured with the temperature capsule is a valid measure for Tc based on the good agreement between intestinal and esophageal temperature. Furthermore, the 95% Bland and Altman limits of agreement were limited to ±0.4°C[26], while no significant bias was found between both measurements[9, 18, 22]. These results suggests that the Tgi is a valid measure for Tc.

Another important aspect of a good Tc/Tgi measurement technique is a high temporal resolution to rapidly monitor changes in Tc. Previous studies have demonstrated that the Tgi measured with the temperature capsule responds more slowly on changes in Tc compared to the esophageal measurement[15, 21, 22], which can be explained due to the low heat capacity of the esophagus and the proximity to the heart[10]. In the esophageal temperature measurement the thermistor is placed at the level of the left atrium[10]. At this level the pulmonary artery and the esophagus are in contact and isothermal[27], which stimulates a fast response time on changes in temperature of the esophageal measurement. In contrast, the intestines and rectum are less perfused compared to the esophagus, resulting in a delay in measuring temperature changes on these anatomical locations. However, the ingestible telemetric temperature capsule has an accuracy of ±0.1°C and is able to measure Tgi every 10 seconds. A previous study reported that core body temperature can raise with a maximum of 1°C every 5 minutes if no heat is removed during exercise[28]. Therefore, the temporal resolution of the temperature capsule is suitable to measure changes in Tgi during exercise. Based on these findings, it can be concluded that the temperature capsule is a reliable and valid technique to measure Tgi. Despite the use of the telemetric temperature capsule in a large number of studies, a clear description about how to use the temperature capsule is missing.

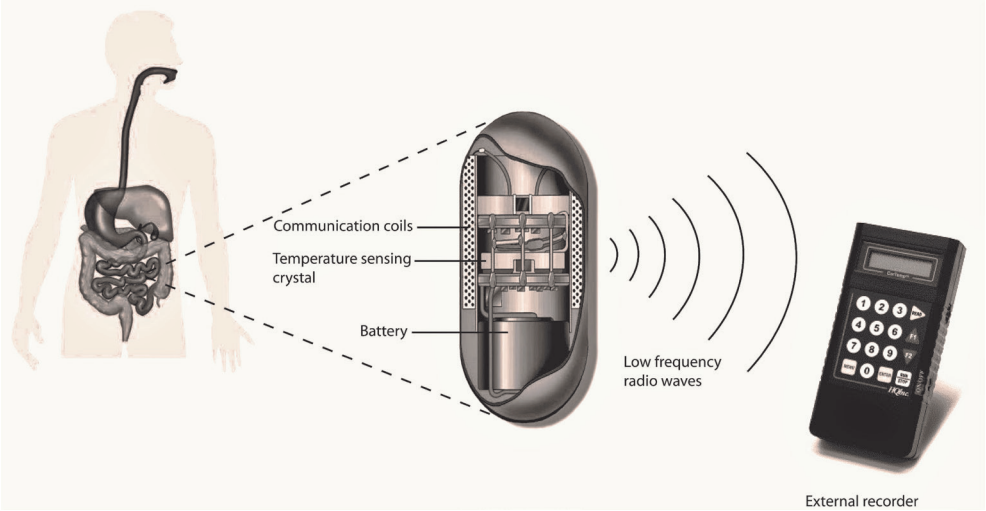


Figure 1. Schematic overview of gastrointestinal temperature measurement.

Table 1. Overview and assessment of techniques to measure core body temperature[9, 10, 12, 15-22]

Method	Advantages	Disadvantages
Mercury thermometer (Mouth or armpit)	- Non-invasive[12,19] - Easy to use[12,19]	- Not suitable during exercise[10,19,22] - Influenced by food and drink intake[10,12,19,22] - Influenced by air temperature[10,12,19,22]
Ear thermometer	- Non-invasive[17,19] - Less accurate on higher temperatures[17]	- Difficult to use[10,12,19] - Influenced by ambient temperature[10,12] - Not suitable during exercise[10,19]
Rectal thermistor	- Very accurate (±0.1°C)[16,19,20]	- Invasive[9,12,19,21,22] - Discomfort during monitoring[17] - Not suitable in field based settings[17]
Esophageal thermistor	- Gold standard[9,10,12,19,21] - Closest agreement with central blood temperature[9,12] - Rapid response to changes in Tc[19,21]	- Invasive[10,12,19,21,22] - Discomfort during monitoring[10,19,21] - Not suitable in field based settings[10,12,17,19]
Telemetric temperature capsule	- Very accurate (±0.1°C)[9,15,17,22] - Non-invasive[16,21] - Suitable in field based settings[9,15,21]	- Expensive[17] - Ingestion ≥6 h before the measurement[9,17]

Figure 2. Ingestible telemetric temperature pill and packing material. Left side: the wrapping material, which contains the temperature pill individual serial and calibration number. Right side: the temperature pill and the magnetic stripe. In this case the temperature pill is not in contact with the magnetic stripe, which means that the battery is activated.

Therefore, the purpose of this study is to provide a detailed description of the measurement protocol using an ingestible telemetric temperature capsule. Secondly, we described the application of the telemetric temperature capsule in two different study protocols, in which a cross-sectional design (measurement every 5 km with a different recorder) and a protocol that continuously records Tgi in individuals are used.

PROTOCOL

The steps described in the following section are in line with and accepted by the medical ethical committee of the Radboud university medical center in Nijmegen, The Netherlands. To our knowledge, 3 different commercial systems of ingestible temperature capsules are currently available for researchers. The user manual of the ingestible temperature capsules is brand-specific, but all systems are suitable for measurements during exercise and under resting conditions.

Exclusion criteria and Subject Instruction

1. Ask subjects in written or verbal form for the exclusion criteria for using the telemetric temperature capsule: 1) body weight below 36.5 kg, 2) obstructive gastro-intestinal disease, 3) history of gastrointestinal surgery, 4) an implanted medical device, and 5) a scheduled MRI scan during the experimental period.
2. Write down the serial and calibration number of the temperature capsule.
3. Instruct the subjects how to use the temperature capsule (see section 2).
4. Give the capsule to the subject together with a short instruction manual, which contains the information shown in section 2. If subjects receive the temperature capsule well ahead of the experiment, remind the subject the day preceding the experiment to ingest the capsule.

Temperature capsule instructions

Instruct the subject to ingest the temperature capsule at least 6 hours prior to the experiment, to avoid any interaction with fluid ingestion. Follow the subsequent steps to ingest the capsule correctly:

1. Remove the magnetic strip from the capsule, to activate the battery and enable measuring.
2. Ingest the temperature capsule preferably with a glass of water to enhance capsule ingestion.
3. Return the capsule wrapping material to the research team, so they can check serial and calibration numbers prior to the start of the experiment.
4. The temperature capsule will leave the body through its natural way (feces) and it can be flushed through the toilet.

Experimental protocol I: Cross sectional mode

Note: In the cross sectional mode it is possible to measure up to 99 subjects simultaneously.

1. Adjust the recorder to the desired settings for the cross sectional measurement prior to the measurement.
    - 1.1 Turn on the recorder, connect the recorder with the computer with a transfer cable and push the 'F2-PC Link' button to enable the recorder to connect with the computer.
    - 1.2 Open the Tc software on the computer, which can be used to define the right settings. Note: The software is supplied by the company with the order of the temperature capsule and recorder.
    - 1.3 To adjust the settings, click on 'Program' in the home screen of the software, and subsequently use the 'open PC link' button to make a connection with the recorder and select the correct settings.
        - 1.3.1 Select the cross sectional measurement mode by selecting 'Sports mode ON'.
        - 1.3.2. Select the correct temperature measurement scale (Celsius or Fahrenheit). Use the 'Write Config to Recorder' button to copy the settings to the recorder.

1.3.3. Add the serial and calibration number of all individual subjects to the external recorder, which enables the option to switch users during the experiment. Push the 'Sensor/Barcode Display' button in the software and add all the serial and calibration numbers. Push the 'Write Sensors to Recorder' button to copy the data to the recorder.

1.3.4. Check the battery of the recorder prior to the measurement, to avoid a discharged battery during the measurement and therefore missing data. Note: Normally, a battery state of 75% is sufficient to measure for >10 hours.

2. Once all preparations are completed and the predefined settings are checked, start the experiment. To do so, return to the home screen of the recorder and use the 'F2-Sport' button to start data acquisition.
3. When Player XX appears on the screen, push the 'Read' button to measure Tgi. Use the 'Read' button again for an extra measurement of Tgi.
4. To switch users, push on the correct number on the recorder and subsequently measure the Tc by pushing the 'Read' button.
5. Stop the data collection by pushing the 'Stop' button.
6. When the measurement is finished, turn off the recorder in the correct way to prevent data loss. To do so, use the 'Enter' button and 'Exit' becomes visible on the home screen. Push the 'F1-Exit' button and the recorder shows 'turn of recorder'. Subsequently, use the power switch to turn off the recorder.
7. Export and store the raw data from the external recorder to a computer (data extraction).

Experimental protocol II: Continuous mode

Note: The continuous mode enables to continuously measure and save the Tgi of an individual subject on a predefined constant time interval, for example every 20 seconds. In the next section, the step sequence used to perform this type of measurement is described.

1. Adjust the recorder to the right settings for the continuous measurement mode prior to the measurement (see experimental protocol I, steps 1.3.1-1.3.4).
2. Select the continuous measurement mode by selecting 'Sports mode OFF'.
3. Select a measuring frequency by adjusting the 'Read Interval' to the right constant time interval (hh:mm:ss), with a minimal sampling interval is 10 seconds.
4. Select the correct temperature measurement scale (Celsius or Fahrenheit). Use the 'Write Config to Recorder' button to copy the settings to the recorder.
5. Check the battery of the recorder prior to the measurement, to avoid a discharged battery during the measurement and therefore missing data. Note: Normally, a battery state of 75% is sufficient to perform a 24 hours measurement.
6. Once all preparations are completed and predefined settings are checked, start the experiment. Start data acquisition by pushing the 'Run' button on the home screen of the recorder.

7. Subsequently, attach the recorder in a waist bag close to the abdominal area of the subject (maximal 30-40 cm between the abdominal area and the recorder) to avoid measurement errors. Note: After the start of the experiment, every predefined time interval a measurement of Tc will be taken. With the 'Read' button extra sampling points can be added.
8. Stop the Tc measurement by pushing the 'Stop' button.
9. Use the 'F1-Exit' button to get the message 'turn off unit' and then use the power switch to turn off the recorder.
10. Export and store the raw data from the external recorder to a computer (see data extraction).

Data Extraction

1. Connect the recorder to the computer to complete data export (section experimental protocol I, step 1.3.1).
2. Open the software and click the 'Download' button in the home screen of the software.
3. Enter a file name and push the 'OK' button. Note: The data will now be stored as a .cvt file, which can be opened using spreadsheet software.
4. Open the data file and visually check the collected data for missing data and outliers. Note: A large decrease or increase of the Tgi (≤1°C) within a short time interval (±1 min) is very unrealistic and may be caused by a disturbance of the radio signal. As a result, the unrealistic data point can be removed for further analysis.
5. Interpolate the missing values by averaging the previous and next valid value. Note: Interpolation of the data is possible with a maximum of three missing values in a row.

REPRESENTATIVE RESULTS

Representative results from our previous work demonstrating the methods are presented in the next section, in which an example of a cross sectional (Figure 3A) and a continuous measurement (Figure 3B) are given.

Cross sectional measurement of Tgi

An example of data from a cross sectional measurement is shown in Figure 3A. After obtaining baseline Tgi, subjects walked 30 km at a self-selected pace. During exercise the Tgi is measured every 5 km as well as directly after finishing the 30 km walking march. Figure 3A represents the results of the Tgi of 4 subjects during the 30 km walking march. The figure demonstrates that the cross sectional mode enables measurement of a group of subjects (up to 99 subjects), using the same equipment.

Continuous assessment of Tgi

In addition to the cross sectional design, the temperature capsule can be used to perform continuous Tgi measurements, in which the Tgi is measured continuously at a high temporal resolution (varying between 10 seconds and 1 hour). In the example presented here, Tgi of 4 healthy adults is measured every minute for 24 hours, to assess the circadian rhythm of the Tgi. All measurements are performed at the home of the participant. After correcting the data for outliers, the average Tgi is plotted in Figure 3B. Even though the number of subjects is very low, the variation in Tc is very low as can be seen from the relatively small error bars. From 09.15 AM Tgi gradually increases during the day until approximately 19.15 PM. Subsequently, the Tgi decreases in the evening and during night, followed by an increase in Tgi in the early morning (from 06.15 AM). The lowest Tgi is found during night time (01.15 AM -06.15 AM). The results of the figure demonstrate that the temperature capsule is a suitable and non-invasive method to continuously measure Tgi in a home-based situation and is able to detect small changes in Tgi.

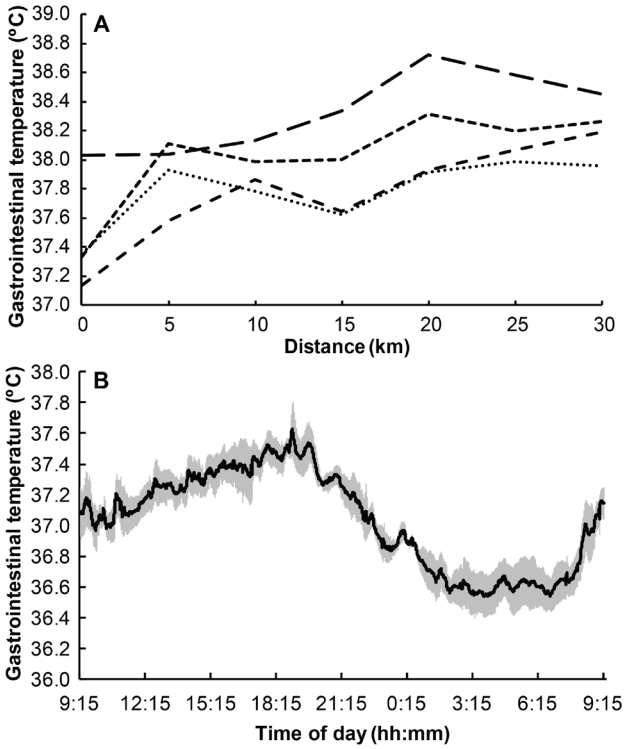


Figure 3. (A) Results of a cross sectional measurement of Tgi during exercise. Data represents Tgi (n=4) measured every 5 km during a 30-km walking march. **(B)** Results of a longitudinal measurement of Tgi (n=4), measured every minute for 24 hours. Data are presented as mean±SE.

DISCUSSION

The ingestible telemetric temperature capsule has the ability to provide a continuous, valid and non-invasive measurement of the Tgi. Furthermore, an advantage of the temperature capsule is the fact that, once ingested, the subjects are unaware of the presence of the capsule in the body or that the measurements are performed. Therefore, this method is easily applicable under resting conditions as well as during exercise, a minimal burden for study subjects, and can therefore be used in field and laboratory settings. Another advantage is the possibility to measure a large group of subjects with only a single recorder.

To ensure an accurate, reliable and safe assessment of Tgi with the ingestible capsule, it is important to follow a number of recommendations. First, the exclusion criteria should be carefully checked, to be sure that the temperature capsule would not be harmful for the subject. Second, it is important to ingest the temperature capsule at least 6 h before the experiment, to avoid any interaction with fluid intake and position in the gastrointestinal tract. In literature different ingestion times prior to data collection are used, ranging from 2 hours[22, 29] to more than 10-12 hours[30, 31]. Interestingly, Sparling *et al.*[32] found no difference in offset between the rectal and capsule temperature during rest and exercise in subjects who swallowed the capsule 3-4 hours prior to data collection and subjects who swallowed the temperature capsule 8-9 hours prior to the measurement. Other studies suggest that an ingestion time of 6 hours is optimal to get a stable measurement of Tgi[9, 18], whilst an ingestion time of 2 hours results in variation in measured Tgi[22, 29]. Wilkinson and colleagues[31] demonstrate that the intake of 250 mL of water influenced the temperature capsule assessment until approximately 5 hours after capsule ingestion. Therefore, a minimum ingestion time of 6 hours preceding the measurement is advised, to avoid any interaction with fluid intake and sensor expulsion prior to data collection. Despite the provided precautions, fluid intake might influence Tgi in some individuals. Therefore, we recommend to visually inspect all raw data for unrealistic Tgi variations. As the maximum Tc increase is 1°C/5 minutes[28], we defined unrealistic variations in Tgi as a decrease or increase of Tgi ≥1°C/minute. These data points may be removed and the missing data can be interpolated using the average of the previous and next value. To ensure valid data collection, the interpolation method may only be used for a maximum of three subsequent data-points. Third, it is of great importance to correctly adjust the serial and calibration number of the temperature capsule in the external recorder. Every temperature capsule is individually calibrated and contains a unique serial and calibration number. The external recorder uses temperature capsule specific serial and calibration numbers to converse the radio signal and measure the Tgi correctly. Thus, without correct numbers the wrong conversion factor is used, resulting in a non-reliable measurement of Tgi.

It is important to notice that this technique has some limitations. First, the cost of the temperature capsule (approximately $50 per capsule) is higher compared to other techniques (tympanic, mouth, or rectum), in particular because the temperature capsule can only be used once. Furthermore, the transit time of the digestive system for a single temperature capsule has to be taken into account when determining the ingestion time preceding the experiment and the total duration of the experiment. A study by Roach *et al.*[33] followed 11 subjects over 7 days, in which they ingested a new temperature capsule as the previous one had left the body. The mean transit time of the digestive system for a single capsule was 27.4 hours (ranging from 4.6 to 82.8 hours). Moreover, the subject with the shortest transit time (4.6 hours) also reported a transit time of 26 hours, whilst the largest within subject difference between transit times was 55 hours. The results of Roach and colleagues[33] suggest a high degree of within- and between subjects variability in transit time of the temperature capsule. The transit time of the gastrointestinal tract is independently influenced by several physiological factors such as gender, age, diet, psycho-behavioral factors (for example short-term anxiety and stress) and physical activity level[34-36]. Therefore, it is important to determine, based on the study protocol, population and variation in transit time, if a continuous measurement over a longer period is suitable to answer the research question. Still, it can be possible that the temperature capsule already left the body prior to the measurement. If this is the case, the measurement must be rescheduled and a new capsule must be ingested 6 hours preceding the experiment. In case of a large amount of missing or unrealistic data it is also advisable to reschedule the experiment to obtain a valid measurement for further processing.

It is important to ensure that the external recorder is close to the temperature capsule to receive the radio signal and convert it to a correct Tgi. The maximal distance between the external recorder and temperature capsule is approximately 0.65 meter, which is sufficient to measure Tgi in humans. In case of obese subjects, it can be recommended to measure Tgi at the posterior instead of the anterior side of the body. Furthermore, it is important to avoid that ≥2 subjects are within close distance (<1.5 meter) of each other, as interference of radio signals may occur. Finally, the storage of the temperature capsules needs special attention to ensure that the sensors stay off and the batteries do not drain. Therefore, it is important to follow the storage guidelines that are provided by the manufacturer and include: I) at least one inch spacing between each sensor; II) never store the temperature capsules near metallic objects; III) preferably keep the temperature capsules in the custom-made foam inserts of the shipping package.

Taken together, the telemetry capsule represents a reliable and valid method to measure the Tgi in both laboratory and field settings. Due to the good measuring accuracy and frequency, the ability to measure in field based situations and the non-invasive character of the temperature measurement (Table 1), the ingestible telemetric temperature capsule is a suitable method to assess Tgi during exercise.

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Chapter 3

Validity and Reliability of the myTemp Ingestible Temperature Capsule

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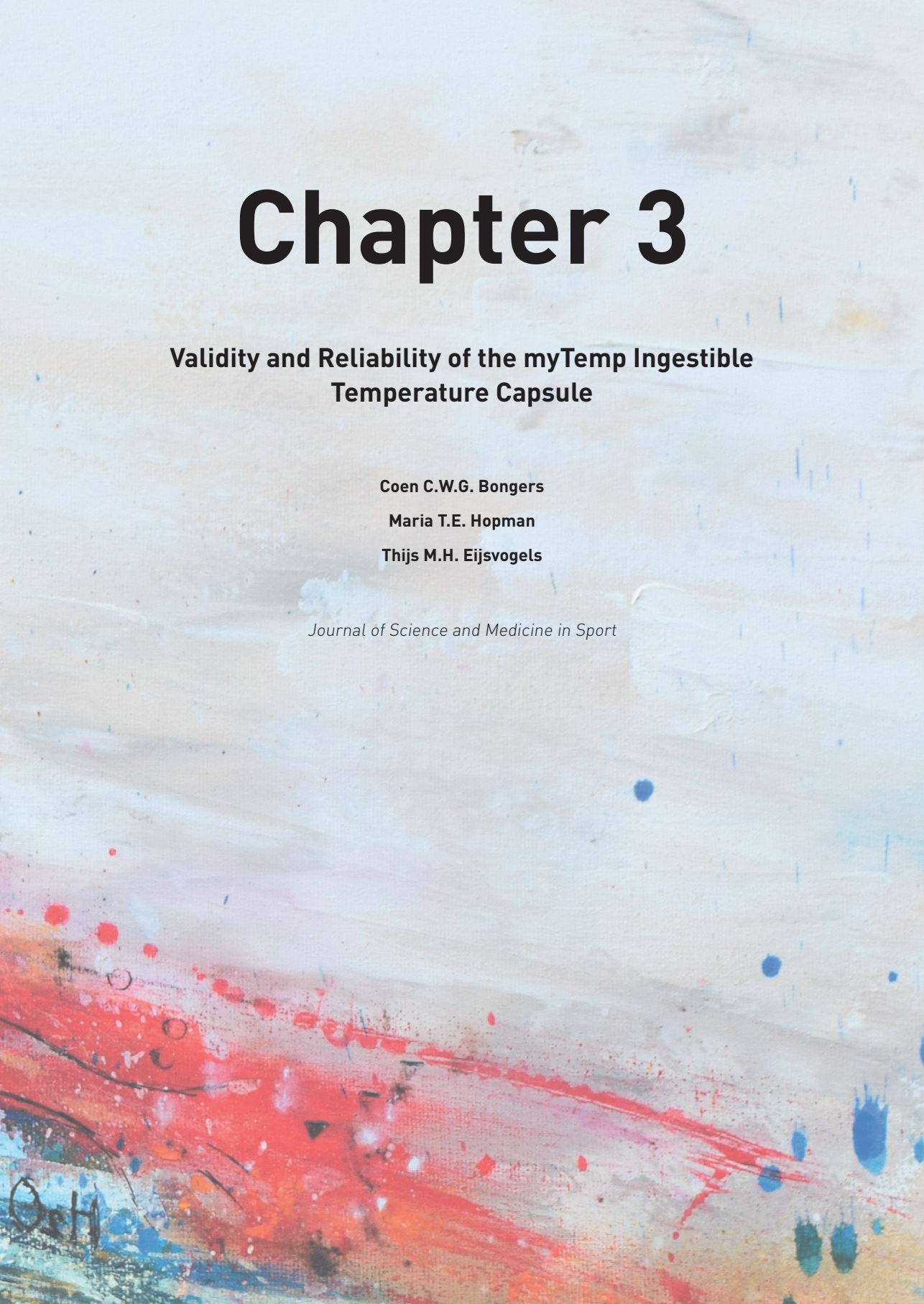
Journal of Science and Medicine in Sport

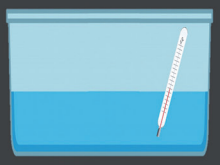


Validity and reliability of the myTemp ingestible temperature capsule

Temperature capsules were tested twice and water bath temperature was measured with four very accurate thermometers

°myTemp

n=15



Measurement protocol

Trial 1

°myTemp

VS

Gold standard

°C

1	Validity
Systematic bias=	-0.001 ± 0.005°C
Limits of agreement =	± 0.011°C

Trial 1

°myTemp

VS

Trial 2

°myTemp

2	Reliability
Systematic bias=	0.004 ± 0.008°C
Limits of agreement =	± 0.015°C

The myTemp capsule is a valid and reliable measure for (water) temperature under controlled circumstances

ABSTRACT

An accurate and non-invasive measurement of core body temperature (Tc) is of great importance to quantify exercise-induced increases in Tc in athletes or to assess changes in Tc in patient populations. The use of ingestible gastrointestinal telemetric temperature capsules is widely accepted as a surrogate marker for Tc, but widespread implementation is lacking due to the high costs of these disposable capsules. A new and cheaper temperature capsule system (i.e. myTemp) was recently introduced. The aim of present study is to determine the validity and test-retest reliability of the myTemp system. Fifteen ingestible temperature capsules (myTemp, Nijmegen, Netherlands) were tested in a highly temperature controlled water bath, in which the water temperature gradually increased from 34°C to 44°C. The study protocol was performed twice for each temperature capsule. The mean difference between myTemp temperature and water bath temperature was -0.001±0.005°C (Limits of Agreement (LOA): ±0.011°C) during Trial 1 (p=0.11) and -0.001±0.006°C (LOA: ±0.012°C) during Trial 2 (p=0.039). Furthermore, an Intraclass Correlation Coefficient (ICC) of 1.00 was found for both trials. A systematic difference between Trial 1 and 2 of 0.004±0.008°C (LOA: ±0.015°C) was found (p<0.001), whereas the ICC between both trials was 1.00 and the standard error of the measurement was 0.005°C. Although we found a systematic bias for the validity (-0.001°C) and reliability (0.004°C), these values can be considered insignificant from a physiological and clinical perspective. Thus, the myTemp ingestible temperature capsule is a valid technique to measure (water) temperature under controlled circumstances.

INTRODUCTION

Core body temperature (Tc) reflects the temperature of the abdominal, thoracic and cranial cavities of the body, which can be measured as an esophageal, pulmonary artery, intestinal and rectal temperature. In healthy individuals Tc is typically regulated between 36.2°C and 37.7°C[1, 2], with a continuous balance between heat production and heat loss[3]. Exercise induces a thermoregulatory burden, as only a minority (*i.e.* 1% - 20%) of the metabolically produced energy can be used as muscle power, whereas the majority (*i.e.* 80% - 100%) of the energy is released as heat[4-6]. The response of heat loss mechanisms to increased heat production during exercise is often delayed and insufficient, which results in heat accumulation and an increase in Tc. The increased Tc may reduce the exercise performance and increases the risk to develop heat-related illnesses such as heat exhaustion and heat stroke during uncompensable heat stroke[7].

In order to protect individuals from severe heat-related illnesses it is important to monitor Tc and anticipate on high Tc levels. Unfortunately, measurement of Tc is difficult. Intra-Pulmonary arterial temperature is currently considered as the gold standard for Tc[8]. However, this Tc measurement is invasive and only applicable in clinical settings. Alternatively, athletes have been using ingestible temperature capsules to wirelessly measure gastrointestinal temperature as a valid surrogate marker of Tc[9-12]. Although several commercial systems are available, current temperature capsules and data recorders are expensive and have important restrictions such as battery lifetime and expiry. A Dutch start-up company, myTemp (www.myTemp.nl), developed and patented a novel ingestible telemetric temperature capsule system, which is cheaper and does not have a battery. However, the precision of this temperature system has not been investigated yet.

Therefore, the aim of present study is to determine the validity and test-retest reliability of the myTemp ingestible temperature capsule. We used an ex-vivo water bath for optimal control of testing conditions. We hypothesized that the myTemp temperature capsule is valid and reliable to measure Tc, with a systematic bias for both parameters <0.1°C.

METHODS

In this ex-vivo experimental study a total of n=15 myTemp ingestible temperature capsules were tested in a custom made highly controlled water bath. The primary outcomes were the validity and test-retest reliability. Two measurements were performed per temperature capsule, using a similar study protocol. All measurements were conducted within a 48 hour period to prevent any drifting due to changes in environmental factors.

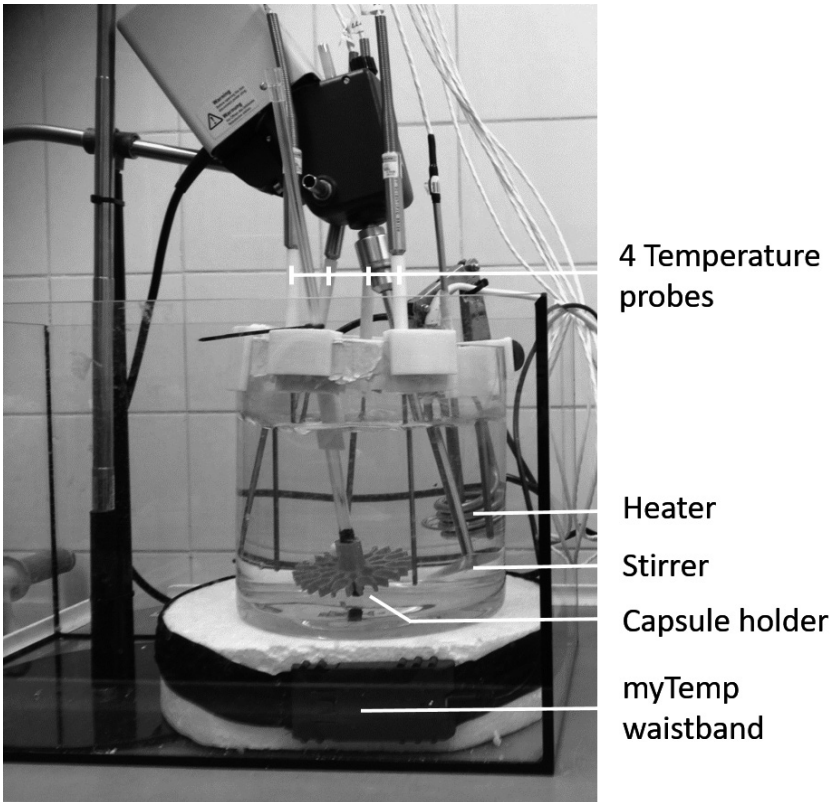


Figure 1. Overview of the study set-up

The myTemp system consists of an ingestible temperature capsule (8 x 20 mm in size, 1.3 g) and a copper-wired waistband which created a magnetic field (myTemp, Nijmegen, Netherlands). The ingestible capsule is activated by the magnetic energy of the waistband under the condition that capsule lies within the circle of the waistband and that the capsule and waistband are within a 30 cm range of each other. The capsule measures its surrounding temperature using a NTC thermistor and sends the data wirelessly to the waistband. Temperature is logged at predefined intervals, which was established at 6 seconds (10 measurements/minute) for the present study. Furthermore, the myTemp capsule is able to record temperature with a detection resolution of 0.01°C.

A thermostat-controlled and distilled water-filled bath (3.5 L) was used in which four highly sensitive wired temperature probes (Fluke Hart Scientific 1529 Chube E-4 Thermometer Readout, Everett, USA) measured temperature up to 0.002°C exactly. The average value of these wired temperature sensors represented the temperature of the water bath. In addition, a heater (Fluke Hart Scientific 2100 Temperature Controller, Everett, USA) and stirrer (Heidolph Instruments D91126, type RZR1,

Schwabach, Germany) system ensured thermal homogeneity of the water bath. A custom made holder prevented the sensor reaching the bottom of the water bath. The myTemp waistband was placed around the water bath with a distance range of 0.2 m from the ingestible temperature capsule. Moreover, the environment in which the equipment was located was also free of signal interference, which may be caused by electromagnetic fields such as computers or phones. An impression of the study setup is presented in Figure 1.

During the measurement protocol the water temperature gradually increased from 34°C to 44°C, mimicking the physiological range between hypothermia (<35°C) and exertional hyperthermia (>40°C). An automated protocol was programmed to perform the stepwise increase in water temperature, resulting in eleven plateaus (34, 35, 36, 37, 38, 39, 40, 41, 42, 43 and 44°C, Figure 2). For each temperature plateau, three conditions had to be achieved before the protocol could proceed:

1. Water bath temperature did not vary >0.02°C during fifty consecutive measurements.
2. Water bath temperature did not vary >0.01°C during two consecutive measurements.
3. The change in heater power did not exceed 4% during two consecutive measurements.

These conditions ensured stability of the water bath temperature and thereby reliable temperature measurements at each point of measurement. The study protocol was performed twice for each temperature capsule (Trial 1 / Trial 2), which allowed us to calculate the validity and test-retest reliability.

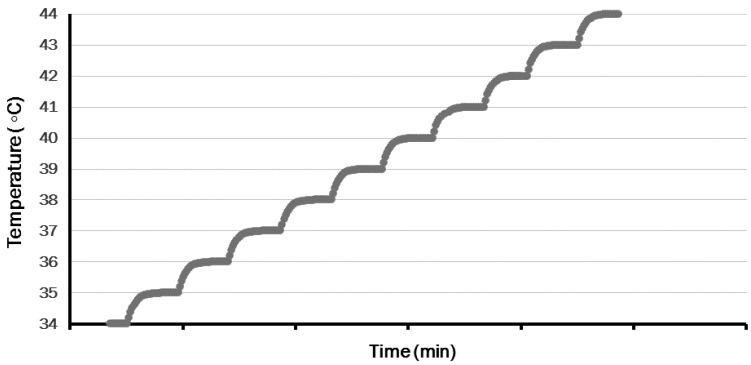


Figure 2. Schematic overview of study protocol with temperature plateaus

The average of the last 25 temperature readings per temperature plateau was calculated and compared to the average water bath temperature over the same period. This resulted in 11 comparisons between the myTemp temperature system and water bath per ingestible temperature capsule. In order to establish the validity of the myTemp capsules, the Bland-

Altman method for assessing the agreement between two methods was used[13]. In short, the mean difference between the myTemp temperature system and water bath was assessed using a one-sample T-test. The systematic bias and accompanying 95% Limits of Agreement (LOA) were derived from the Bland-Altman plot[13]. Furthermore, a bi-variate correlation plot was constructed for the average temperature of the myTemp capsules and the average water bath temperature. The Intraclass Correlation Coefficient (ICC) was calculated for the average of all 15 capsules, to determine the inter-measure agreement[14]. The Standard Error of Measurement (SEM) was calculated based on the standard deviation (SD) of the difference between myTemp and water bath temperature[15]. Furthermore, we conducted a Repeated Measures ANOVA to determine whether the accuracy of the myTemp capsule (defined as Δ water bath - myTemp temperature) was different across the physiological temperature range.

A similar approach was used to determine the test-retest reliability. A Bland-Altman plot was constructed to determine the agreement of the myTemp ingestible capsule data between the first and second measurement. Furthermore, a bi-variate correlation plot was created and the ICC for agreement and 95% LOA were reported. The SEM was determined using the SD of the difference of myTemp temperature between Trial 1 and 2. All statistical analyses were performed using SPSS Statistics (Version 20), in which the level of significance was set at $p<0.05$. The mean difference was reported as mean difference ± SD, unless indicated otherwise.

RESULTS

All tests were performed successfully and data was collected within 4 weeks. No signs of data interference were observed and no outliers were detected.

Validity. The mean difference between the myTemp temperature and the water bath temperature was -0.001±0.005°C during the validation Trial 1, and no evidence of a systematic bias was found ($p=0.11$) (Figure 3A). Furthermore, the LOA were ±0.011°C. During validation Trial 2, a similar mean difference was observed (-0.001±0.006°C), but this appeared to be statistically significant ($p=0.039$) (Figure 3C). Additionally, the LOA of Trial 2 were ±0.012°C. An ICC of 1.00 was found between myTemp and water bath temperature for validation Trial 1 and 2 (both p-values <0.001, Figure 3B & 3D). The SEM between myTemp and water bath temperature was 0.004°C for both trials. A repeated-measurements ANOVA revealed that the mean difference between the myTemp system and water bath temperature drifted across plateaus ($p<0.001$), with a minor overestimation (0.002°C) at low temperatures (34-38°C) and a minor underestimation (-0.003°C) for higher temperatures (39-44°C) (Table 1).

Test-Retest Reliability. The mean difference between trial 1 and trial 2 was (0.004±0.008°C, LOA= ±0.015°C, Figure 4A), which appeared to be a significant bias (p<0.001). Nevertheless, we found a good agreement between both trials based on the ICC of 1.00 for both the comparison between myTemp temperature Trial 1 and 2, and the comparison between the mean myTemp and water bath temperature for Trial 1 and 2 (both p-values <0.001, Figure 4B). A SEM of 0.005°C was found between myTemp temperature Trial 1 and 2.

Table 1. Difference between water bath and myTemp temperature (Δ Water bath – myTemp) over the temperature range. Data are presented as averages from n=15 capsules measured during two separate trials.

	Trial 1		**Trial 2**	
Target Temperature (°C)	Δ Water bath - myTemp (°C)	p-value*	Δ Water bath - myTemp (°C)	p-value*
34	0.003 ± 0.005	0.06	0.005 ± 0.005	**0.002**
35	0.002 ± 0.003	0.06	0.002 ± 0.004	**0.042**
36	0.002 ± 0.005	0.15	0.003 ± 0.004	**0.013**
37	0.002 ± 0.005	0.20	0.002 ± 0.005	0.10
38	0.002 ± 0.005	0.17	0.001 ± 0.005	0.58
39	-0.002 ± 0.005	0.12	-0.002 ± 0.006	0.16
40	-0.005 ± 0.006	**0.006**	-0.005 ± 0.006	**0.008**
41	-0.003 ± 0.005	**0.048**	-0.004 ± 0.005	**0.007**
42	-0.002 ± 0.005	0.15	-0.004 ± 0.006	**0.017**
43	-0.002 ± 0.003	**0.038**	-0.003 ± 0.004	**0.012**
44	-0.003 ± 0.006	**0.042**	-0.005 ± 0.004	**<0.001**

Repeated measures ANOVA revealed a significant difference in Δ water bath – myTemp temperature across the temperature range (p<0.001 for both Trial 1 and Trial 2). * Represents the p-value for the post-hoc one sample t-test analysis.

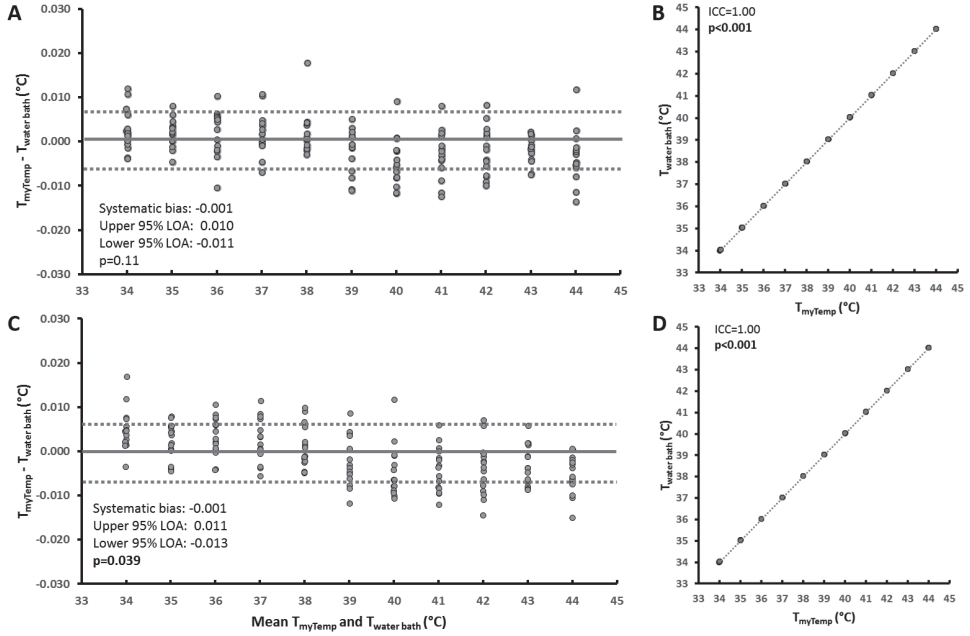


Figure 3. Bland-Altman plot of myTemp versus water bath temperature for Trial 1 **(A)** and Trial 2 **(C)**. Data were presented as mean difference (solid line) ± LOA (dotted line). Furthermore, a bi-variate correlation was plotted for both Trial 1 **(B)** and 2 **(D)**, in which the myTemp temperature (x-axis) was plotted versus the water bath temperature (y-axis). The negligible systematic bias in both trials suggests a good agreement between myTemp and water bath temperature, while the ICC of 1.00 suggests an excellent accuracy of the myTemp system.

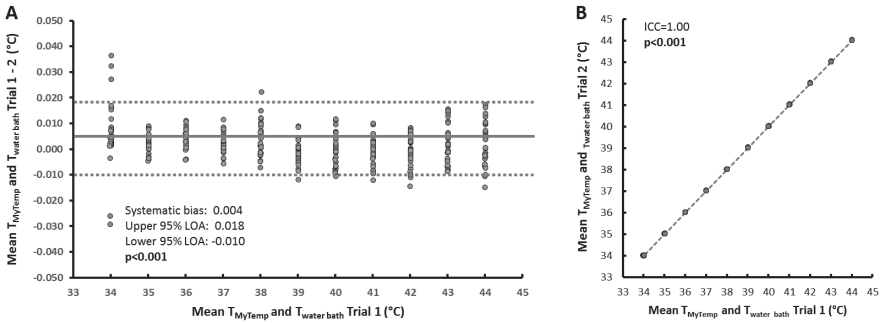


Figure 4. Bland-Altman plot of Trial 1 versus Trial 2 (A). Data were presented as mean difference (solid line) ± LOA (dotted line). A significant, but physiological and clinical negligible, systematic bias was found between myTemp and water bath temperature. Furthermore, the bi-variate correlation plot (B) of Trial 1 (x-axis) and Trial 2 (y-axis) suggests an excellent agreement between Trial 1 and Trial 2.

DISCUSSION

This study examined the validity and reliability of the myTemp ingestible temperature capsule as a method to assess temperature. We found that the myTemp system is a valid technique to measure (water) temperature under controlled circumstances, evidenced by a low LOA (± 0.011) and a small mean difference (-0.001±0.005°C) between water bath and myTemp temperature. Furthermore, an excellent test-retest reliability (LOA= ±0.015°C, ICC=1.00 and SEM=0.005°C) was found, in combination with a small, but significant, mean difference (0.004±0.008°C) between Trial 1 and Trial 2. These findings suggest that the myTemp system is useful for (exercise) scientists and clinicians to accurately measure temperature in a non-invasive way.

Criteria have been formulated to determine the validity of novel measurement techniques. Preferably, the accuracy of the measurements is characterized by a I) low systemic bias, II) narrow 95% LOA, III) high ICC with the reference temperature, and IV) low SEM[16]. For assessment of Tc, a thermometer must have an accuracy of approximately 0.1°C without influences of environmental factors[17], in combination with an acceptable level of agreement, described as a systemic bias <0.1°C and 95% LOA within ±0.4°C[9]. Based on the low systemic bias (-0.001°C) and narrow LOA (±0.011°C) we can conclude that the myTemp capsule system is a valid method to assess (water) temperature under controlled circumstances. Although we found a significant systemic bias of -0.001°C between the myTemp and water bath temperature in Trial 2, the difference between both measurements complies easily with the acceptable level of agreement and is clinically and physiologically negligible. Moreover, a change in Tc of ±0.1°C has been established as physiologically and clinically relevant[17]. Furthermore, the mean difference between the myTemp and water bath temperature drifted across the physiological range (34-44°C), with a negligible overestimation (0.002°C) at low temperatures (34-38°C) and a negligible underestimation (-0.003°C) for higher temperatures (39-44°C) However, the drifted response throughout the physiological range complies with the criteria for an acceptable level of agreement, in which a maximal difference of 0.010°C was found between target temperature 34°C and 44°C (Table 1). As previous studies have demonstrated that the gastrointestinal temperature is a valid and reliable surrogate measure for Tc[9, 11, 18], these results suggests that the myTemp temperature capsule is a valid method to assess Tc in different circumstances.

A reliability of 0.004°C was found, accompanied by a narrow LOA (±0.015°C), high ICC (1.00), and low SEM (0.005°C). These findings suggest that the myTemp temperature capsule has a very good test-retest reliability. Typically, an ICC of 0.70 is considered as acceptable, with higher values representing a better reliability[19]. An ICC of 1.00, such as found in current study, suggest that the error variance between both measurements is negligible compared to the normal variance[20]. The significant systematic bias between Trial 1 and 2 is physiologically negligible

and is well below the acceptable accuracy level of 0.1°C. Therefore, the myTemp temperature sensor is reliable method to perform repeated core body temperature measurements.

This is the first ex-vivo validation study that compares a telemetric temperature capsule system with the average of four highly sensitive temperature probes across the whole physiological Tc range (34°C-44°C). In previous validation studies, water bath temperature measured by temperature capsule systems was compared with the water bath temperature measured with less accurate thermometers such as a rectal probe[16] or a mercury thermometer[10]. They found a systemic bias of 0.17±0.15°C (LOA = 0.30°C), 0.23±0.17°C (LOA = 0.34°C), and 0.27±0.09°C for the VitalSense[16], e-Celsius[16] and CorTemp[10] temperature capsule system respectively, which are markedly higher than the bias found for the myTemp system. The higher systemic bias found in these studies can be explained by a less accurate temperature capsule system as well as by the normal variance of a rectal probe or mercury thermometer. Moreover, other instruments to measure Tc (i.e. esophageal or rectal probe, tympanic thermistor) did neither have an accuracy lower than 0.1°C[21]. Therefore, the myTemp temperature capsule may become the preferred method to accurately assess Tc.
The strengths of current study are the controlled study protocol, with four highly sensitive wired temperature probes (up to 0.002°C), a stepwise increase in water bath temperature, and the criteria for reaching a stable plateau phase. As in our study, the resolution of the myTemp system was 0.01°C, which is physiologically sufficient for in-vivo measurements in sport and exercise sciences. However, we have to take a limitation into account. Within this study, we only examine the validity and reliability of the myTemp system in controlled ex-vivo conditions. Therefore, it will be important to examine whether the myTemp system demonstrates a similar accuracy and reliability in less controlled in-vivo circumstances. Furthermore, the study design of our study should be repeated for the other temperature capsule systems, in order to point out the most accurate system to measure the intestinal temperature.

CONCLUSION

Based on the low systemic bias, narrow 95% LOA, high ICC with the reference temperature, and low SEM, we believe that the myTemp ingestible temperature capsule is a valid and reliable technique to measure (water) temperature under controlled circumstances. Moreover, the consistent low systemic bias and low limits of agreement suggest that the myTemp capsule should not be calibrated prior to usage. Future studies investigating the myTemp system in an ex-vivo as well as in-vivo setting in lab- and field conditions are needed to confirm the superiority of this novel temperature system compared to other commercially available products.

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Chapter 4

Validity, Reliability and Inertia of Four Different Temperature Capsule Systems

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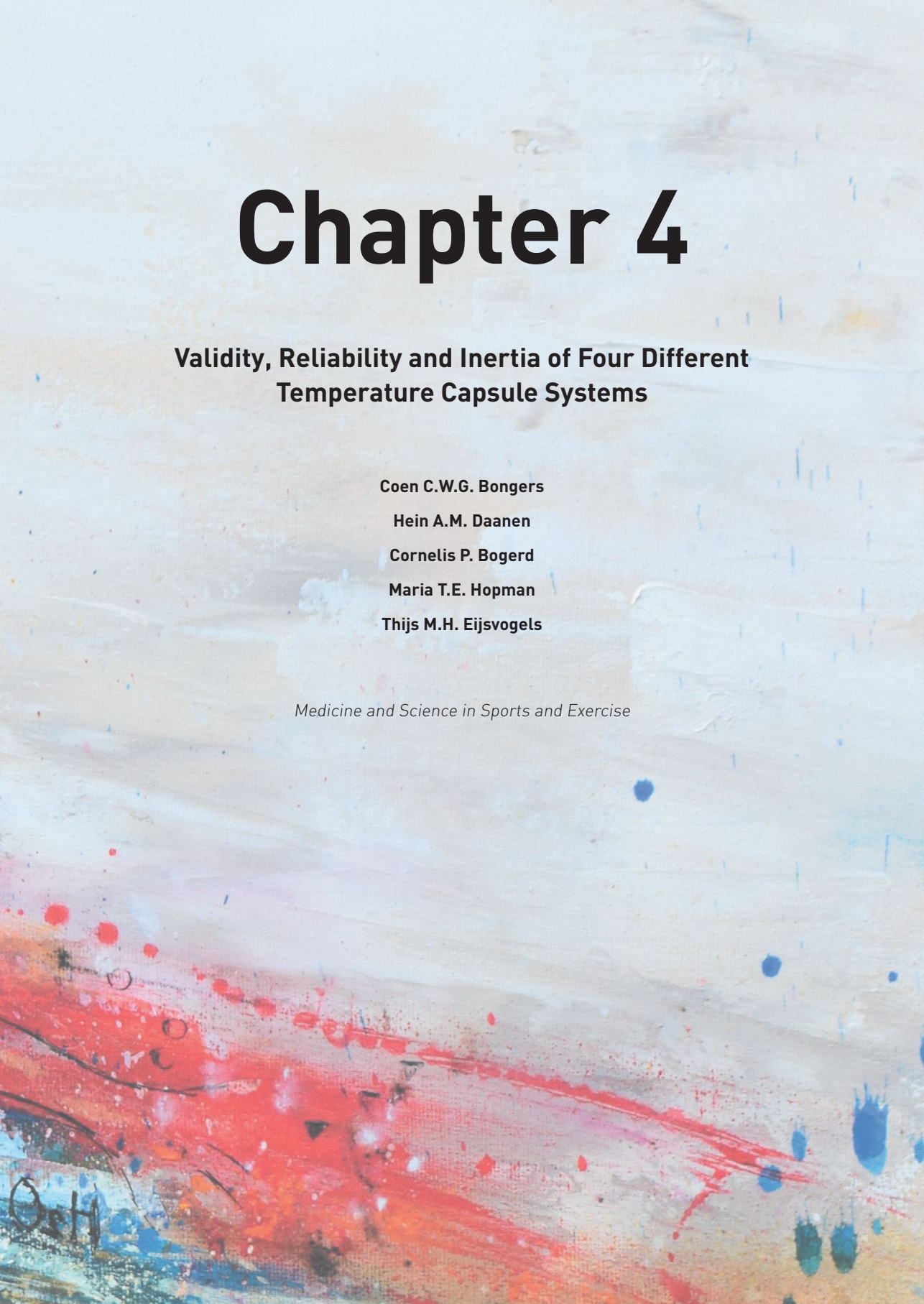


Validity, Reliability and Inertia of four different temperature capsule systems

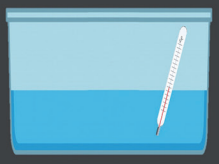
Temperature capsules (n=15) were tested three times and water bath temperature was measured with very accurate thermometers

CorTemp

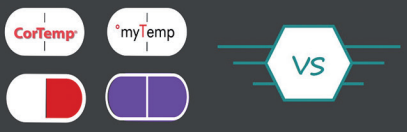
e-Celsius

myTemp

VitalSense



Measurement protocol



Gold standard

1 Validity

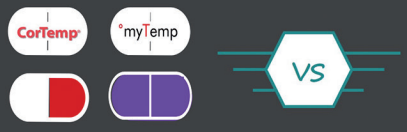
CorTemp	=	0.077 ± 0.040°C
e-Celsius	=	-0.081 ± 0.055°C
myTemp	=	-0.003 ± 0.006°C
VitalSense	=	-0.017 ± 0.023°C

Trial 1

Trial 2

2 Reliability

CorTemp	=	0.017 ± 0.083°C
e-Celsius	=	-0.007 ± 0.033°C
myTemp	=	0.001 ± 0.008°C
VitalSense	=	0.002 ± 0.014°C



Response time

3 Inertia

CorTemp	=	25 ± 4 seconds
e-Celsius	=	21 ± 13 seconds
myTemp	=	19 ± 2 seconds
VitalSense	=	39 ± 6 seconds

An excellent validity, test-retest reliability and inertia was found for all capsule systems between 36°C and 44°C

ABSTRACT

Telemetric temperature capsule systems are wireless, relatively non-invasive and easily applicable in field conditions, and have therefore great advantages for monitoring core body temperature. However, the accuracy and responsiveness of available capsule systems have not been compared previously. Therefore, the aim of this study was to examine the validity, reliability and inertia characteristics of four ingestible temperature capsule systems (i.e. CorTemp, e-Celsius, myTemp and VitalSense). Ten temperature capsules were examined for each system in a temperature controlled water bath during three trials. The water bath temperature gradually increased from 33°C to 44°C during Trial 1 and 2 to assess the validity and reliability, and from 36°C to 42°C in Trial 3 to assess the inertia characteristics of the temperature capsules. A systematic difference between capsule and water bath temperature was found for CorTemp (0.077°C±0.040°C), e-Celsius (-0.081°C±0.055°C), myTemp (-0.003°C±0.006°C) and VitalSense (-0.017°C±0.023°C) ($p<0.010$), with the lowest bias for the myTemp system ($p<0.001$). A systematic difference was found between Trial 1 and Trial 2 for CorTemp (0.017°C±0.083°C, $p=0.030$) and e-Celsius (-0.007°C±0.033°C, $p=0.019$), whereas temperature values of myTemp (0.001°C±0.008°C) and VitalSense (0.002°C±0.014°C) did not differ ($p>0.05$). Comparable inertia characteristics were found for CorTemp (25±4 sec), e-Celsius (21±13 sec) and myTemp (19±2 sec), while the VitalSense system responded more slowly (39±6 sec) to changes in water bath temperature ($p<0.001$). Although differences in temperature and inertia were observed between capsule systems, an excellent validity, test-retest reliability, and inertia was found for each system between 36°C and 44°C after removal of outliers.

INTRODUCTION

Major sport events are increasingly organized in extreme environmental conditions, making it more important for athletes to perform well in hot and cold ambient conditions and to monitor their core body temperature from a safety perspective (Tc). Exercise-induced increases in metabolic heat production[1, 2] are known to induce a major physiological challenge to the thermoregulatory system[1, 3]. A disbalance between heat production and heat loss causes the core body temperature (Tc) to rise, which may lead to the development of exertional hyperthermia (Tc>40°C), heat related illnesses (i.e. heat exhaustion/heat stroke) and/or a reduction of athletic performance[2, 4, 5]. Alternatively, exercise in cold environments could lead to rapid heat loss due to conduction (water), convection (wind) and radiation, which may contribute to the development of hypothermia[6]. Hence, accurate assessment of an athlete's Tc is important to assess the presence and magnitude of thermoregulatory strain and to select and apply appropriate cooling or heating techniques for preservation of health and exercise performance[7-9].

The gastrointestinal temperature, measured with ingestible temperature capsules, has been established as a valid surrogate marker for Tc[10-12]. Temperature capsule systems are wireless, relatively non-invasive and easily applicable in field based conditions. Although the validity of these temperature capsule systems have been examined[11, 13, 14], different study designs were applied and a substantial variation in accuracy was found (*i.e.* -0.001°C - 0.27°C). Hence, it is essential to determine which capsule system is superior for assessment of Tc in field conditions.

The aim of this study was to examine the validity, reliability and inertia characteristics of four commercially available ingestible telemetric temperature capsule systems (*i.e.* CorTemp, e-Celsius, myTemp and VitalSense) in well controlled ex-vivo circumstances using a water bath. Data from this study provide insight in which telemetric capsule system has the most favorable characteristics for Tc assessment, which could enable researchers and trainers to select the best temperature sensor for their scientific study and/or daily practice.

METHODS

Experimental design

Four different ingestible telemetric temperature capsule systems (CorTemp, e-Celsius, myTemp and VitalSense) were tested in a custom made accurately controlled water bath. The primary outcomes were the validity, test-retest reliability and inertia characteristics of the capsule systems. A total of 10 temperature capsules from a single production batch of each

telemetry system were tested during three separate trials. The first and second trial consisted of a similar study protocol and was used to assess the validity and test-retest reliability. The third trial adopted a different protocol and was used to examine the inertia characteristics of the temperature capsules. To reduce any bias caused by environmental factors and to ensure that the capsule systems were evaluated in comparable circumstances, a single capsule for each capsule system was used simultaneously in each trial.

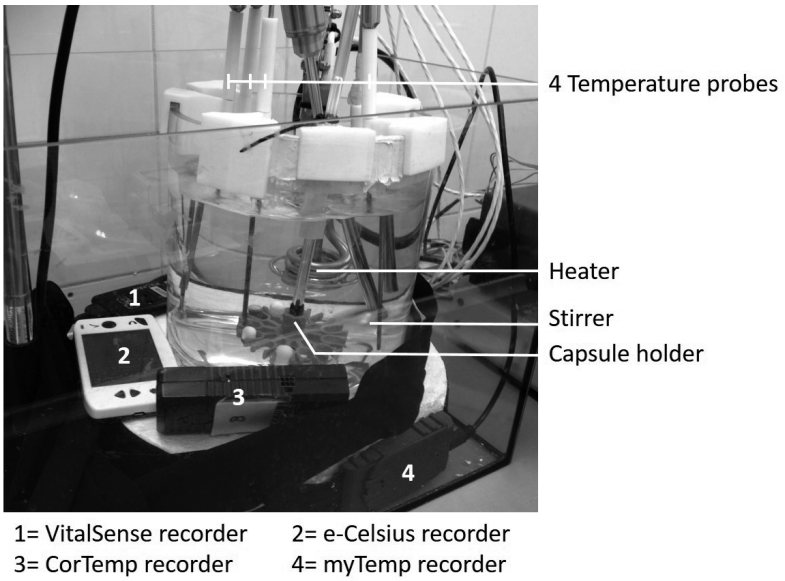


Figure 1. Overview of the experimental setup

Experimental Setup

An overview of the experimental setup is presented in Figure 1. A thermostat-controlled and distilled water-filled bath (3.5 L) was used in which four highly sensitive and calibrated wired temperature probes (1529 Chube E-4 Thermometer Readout Thermistor, Fluke Hart Scientific, Everett, USA) measured temperature up to 0.00035°C exactly. The average value of these wired temperature sensors represented the temperature of the water bath. In addition, a heater (Fluke Hart Scientific 2100 Temperature Controller, Everett, USA) and stirrer (Heidolph Instruments D91126, type RZR1, Schwabach, Germany) system ensured thermal homogeneity of the water bath. A custom made holder prevented the sensor reaching the bottom of the water bath or coming into contact with another sensor. The external monitors of each of the telemetric capsule systems were placed around the water bath within a distance range of 0.2 m.

Study protocol

Prior to each experiment, the sensors and external monitors were synchronized to ensure that the measurements occurred simultaneously. In the validity and reliability measurements the water bath temperature gradually increased from 33°C to 44°C, exceeding the physiological range between hypothermia (<35°C) and exertional hyperthermia (>40°C). An automated protocol was programmed to induce a stepwise increase in water bath temperature, resulting in twelve temperature plateaus (33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 and 44°C). For each temperature plateau, three conditions had to be achieved before the protocol could proceed: 1) water bath temperature did not vary >0.02°C during fifty consecutive measurements (5 minutes), 2) the average value of the four independent probes did not vary >0.01°C during two consecutive measurements, and 3) the change in heater power did not exceed 8% during two consecutive measurements. These conditions ensured stability of the water bath temperature and thereby reliable temperature measurements at each point of measurement. The study protocol was performed twice for each temperature capsule (Trial 1/Trial 2), which allowed us to calculate the validity and test-retest reliability. The water bath temperature was measured every 6 seconds.

In the inertia experiment the water bath temperature gradually increased from 36°C to 42°C. At every temperature threshold (36, 37, 38, 39, 40, 41 and 42°C) the water bath temperature was stabilized for five minutes. Then, the water bath temperature increased by 1°C in a timeframe of five minutes. This timeframe was constructed to mimic the increase in Tc during high intensity exercise in hot ambient conditions, if no heat can be removed from the body[2]. This study protocol allowed us to calculate the time delay of the temperature measured by the temperature capsule compared to the actual temperature of the water bath during the stepwise heating phase. This time delay is defined as the inertia of the temperature capsule.

Telemetric temperature capsule systems

Characteristics of the ingestible telemetric temperature capsule systems are shown in Table 1. All capsule systems used an external wireless recorder to receive the signal from the temperature capsule via a specific radio frequency. The temperature capsules of CorTemp (HQ Inc., Florida, USA), e-Celsius (BodyCap, Caen, France) and VitalSense (Philips Respironics, Bend, Oregon, USA) were delivered in standby modus and had to be activated before use. The myTemp (myTemp, Nijmegen, Netherlands) capsule is automatically activated by the external recorder, which is also the power supply for the temperature capsule. All temperature capsules were activated directly prior to Trial 1. Furthermore, all measurements were performed in accordance with the manual of the individual capsule systems and the highest sample frequency was used throughout the protocol. The external recorders of all capsule systems stored the data, which were exported to a computer for further analysis using the latest version of available software.

Table 1. Physical and technical characteristics of the telemetric capsule systems

	CorTemp	e-Celsius	myTemp	VitalSense
Capsule characteristics				
Length (mm)	22.4	17.7	20.0	23.0
Diameter (mm)	10.9	8.9	8.0	8.7
Weight (g)	2.8	1.7	1.3	1.5
Operating range (°C)	30 to 45	0 to 50	30 to 45	-10 to 60
Accuracy (°C)	0.27[11]	0.23[13]	0.001[14]	0.17[13]
Battery lifetime	7-10 days	20 days	Infinite	10 days
Power supply	Silver-oxide battery	Zinc-silver oxide battery	Self-induction	Battery
Sample frequency	Adjustable	Fixed	Adjustable	Fixed
Lowest sample rate (sec)	10	~30	6	~15
Software version	CorTrack II	e-Performance manager (v01.01.00.0C)	myTemp Manager (v01.08)	Equivital Manager (v1.2.39.4600)

Data processing and Statistical Analysis

The average capsule temperature during the final 150 seconds of each temperature plateau was calculated per telemetric system. Due to differences in sample rate, capsule temperature reflected the average of n=25 consecutive measurements for myTemp, n=15 for CorTemp, n=6 for e-Celsius, and n=10 for VitalSense. Average capsule temperature and water bath temperature were compared for each temperature plateau (33-44°C). Outliers were defined as observations with a difference >1°C between consecutive measurements and were excluded from further analysis. Furthermore, we addressed the number of measurements with a difference between consecutive data points between 0.2°C and 1.0°C to get more insight into the consistency of the data.

In order to establish the validity, the Bland-Altman method for assessing the agreement between two methods was used[15]. In short, the mean difference (=systematic bias) between the temperature capsule and water bath was assessed using a one-sample T-test. The systematic bias and accompanying 95% Limits of Agreement (LOA) were derived from the Bland-Altman plot[15]. Furthermore, the Intraclass Correlation Coefficient (ICC) was calculated for the average of all 10 capsules, to determine the inter-measure agreement[16]. The Standard Error of Measurement (SEM) was calculated based on the standard deviation (SD) of the difference between temperature capsules and water bath temperature[17]. Furthermore, we conducted a Repeated Measures ANOVA to determine whether the accuracy of the capsule systems was different across temperature plateaus (*i.e.* 33-44°C). Differences in accuracy across capsule systems were examined using one-way ANOVA. A similar approach was used to determine the test-retest reliability.

Inertia was assessed as the time delay of the telemetric capsule to reach the same temperature as the water bath after a sudden temperature increase. Inertia was determined at 50% (P50) and 90% (P90) of the increase to each temperature plateau, and the time at which the first observation of the capsule and the water bath exceeded the P50 or P90 temperature was taken. Subsequently, the time to reach P50 and P90 of the capsule system was compared with the time of the water bath to reach P50 and P90, and was defined as the time delay (inertia). As the time delay may be influenced by the accuracy and sample frequency of the capsule, we applied two different correction methods: 1) the systematic bias of the telemetric capsule (i.e. sensitivity data) was subtracted from the recorded values, 2) temperature data was interpolated between subsequent samples to determine the exact time at which P50 and P90 were exceeded. Inertia characteristics were presented as: I) raw data, II) corrected for differences in accuracy, and III) corrected for differences in accuracy and sample frequency. To examine whether there was an inertia difference per temperature plateau across telemetric capsule systems, a two-way repeated measures ANOVA was performed. One-way ANOVA was used to assess the differences in inertia characteristics at P50 and P90 between the four telemetric capsule systems. Furthermore, time constants of the systems response were determined by exposing a single capsule three times to a step change in temperature between two water baths of 7°C (30 – 37°C). Differences in the systems sampling rates did not allow a very precise determination, however by interpolation of the data the time constants can be determined.

All statistical analyses were performed using SPSS Statistics (Version 20), in which the level of significance was set at $p<0.05$. The systematic bias was reported as mean difference ± SD, unless indicated otherwise.

RESULTS

Missing data and outliers. A total of 40 temperature capsules were investigated: 10 sensors per telemetric capsule system. We experienced difficulties with the activation of n=4 VitalSense telemetric capsules, although the provided instructions were carefully followed. Moreover, n=1 of these VitalSense temperature capsules could not be activated at all and 1 temperature capsule stopped measuring after 43°C during Trial 2, meaning that data of the temperature plateau of 44°C is not reported for that temperature capsule. As a result, data from 39 temperature capsules was used for our analyses.

In 6 out of 9 VitalSense temperature capsules, data was randomly missed throughout the protocol (Trial 1 + 2), representing 1.0% of the total data. n=2 CorTemp capsules and n=1 e-Celsius capsule randomly missed 0.1% of the data, whereas no missing data was reported for the myTemp system (Table 2). The CorTemp system appeared to be the only system with

outliers ($\Delta T_{capsule}$ >1°C), which was randomly present in 4.0% of the total data, ranging from a difference of 1°C to 62.1°C. CorTemp also showed error measurements (0.2°C < $\Delta T_{capsule}$ <1°C) in 4.4% of the total data, whereas these error measurements were not present in the other systems. Outliers and error measurements were both found in all CorTemp capsules.

Table 2. Missing data and outliers

		CorTemp	e-Celsius	myTemp	VitalSense
Trial 1	Missing data	0.1%	0%	0%	0.4%
	Outliers > 1°C	3.1%	0%	0%	0.1%
	Error measurements 0.2°C < $\Delta T_{capsule}$ < 1°C	4.1%	0%	0%	0%
Trial 2	Missing data	0.1%	0.3%	0%	1.5%
	Outliers > 1°C	4.9%	0%	0%	0.3%
	Error measurements 0.2°C < $\Delta T_{capsule}$ < 1°C	4.7%	0%	0%	0%

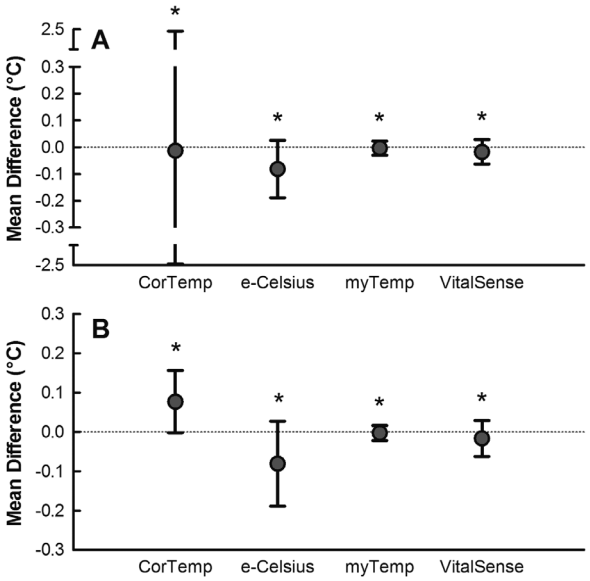


Figure 2. Raw data **(A)** and data after outlier removal **(B)**. Mean difference between capsule and water bath temperature for the capsule systems. Data were presented as mean difference ± LOA. * indicates a significant systematic bias.

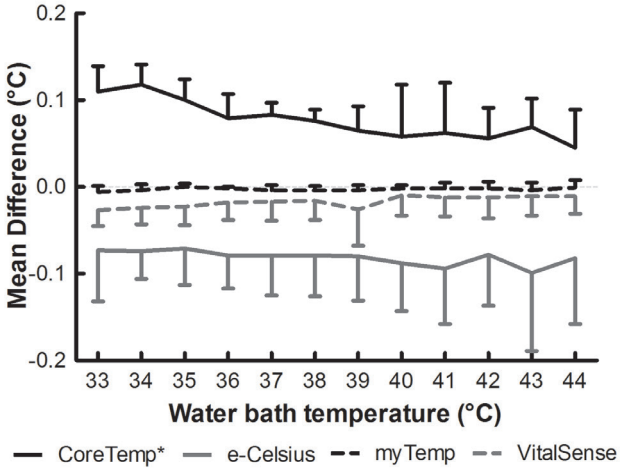


Figure 3. An overview of the mean difference between capsule and water bath temperature for the twelve discrete temperature plateaus. A separate line was plotted for each temperature capsule system. Data were presented as mean difference ± SD, and * represents a drifted response over the temperature plateaus.

Table 3. Validity of the four temperature capsule systems

		CorTemp	e-Celsius	myTemp	VitalSense
Trial 1	ICC – raw data	0.94	1.00	1.00	1.00
	ICC – after outlier removal	1.00	1.00	1.00	1.00
	SEM (°C) – raw data	0.836	0.039	0.005	0.017
	SEM (°C) – after outlier removal	0.028	0.039	0.005	0.017
Trail 2	MD (°C) – raw data	-0.154	-0.073	-0.002	-0.018
	MD (°C) – after outlier removal	0.061	-0.073	-0.002	-0.018
	LOA (°C) – raw data	1.466	0.105	0.013	0.037
	LOA (°C) – after outlier removal	0.167	0.105	0.013	0.037
	ICC – raw data	0.98	1.00	1.00	1.00
	ICC – after outlier removal	1.00	1.00	1.00	1.00
	SEM (°C) - raw data	0.529	0.038	0.005	0.013
	SEM (°C) - after outlier removal	0.060	0.038	0.005	0.013

ICC= Intraclass Correlation Coefficient, SEM= Standard Error of the Measurement, MD= Mean Difference, LOA= Limits of Agreement.

Validity. After exclusion of outliers, mean differences between capsule and water bath temperature for Trial 1 were 0.077±0.040°C (CorTemp), -0.081±0.055°C (e-Celsius), -0.003±0.006°C (myTemp) and -0.017±0.023°C (VitalSense) (Figure 2), which were significantly different from zero (all p-values≤0.01). Additionally, the myTemp system demonstrated the smallest mean difference, followed by VitalSense, CorTemp and e-Celsius ($p_{capsule\ system}$<0.001). The 95% LOA were ±0.079°C (CorTemp), ±0.108°C (e-Celsius), ±0.013°C (myTemp) and ±0.046°C (VitalSense). The SEM was 0.028°C for CorTemp, 0.039°C for e-Celsius, 0.005°C for myTemp and 0.017°C for the VitalSense system. All capsule systems demonstrated an excellent agreement between capsule and water bath temperature based on the significant ICC of 1.00 (all p-values <0.05). The data of Trial 2 revealed similar outcomes with respect to the mean differences, LOA, SEM and ICC (Table 3). A repeated-measures ANOVA indicated that the mean difference between the e-Celsius, myTemp and VitalSense system and water bath temperature did not drift across temperature plateaus (p<0.05). In contrast, a significant decrease in mean difference was found across increasing water bath temperatures for the CorTemp system (p=0.002, Figure 3).

Test-retest reliability. Mean difference between Trial 1 and Trial 2 appeared to be significantly different from zero for CorTemp (0.017±0.083°C, LOA= ±0.162°C, p=0.030) and e-Celsius (-0.007±0.033°C, LOA= ±0.064°C p=0.019) (Figure 4). For myTemp (0.0001±0.008°C, LOA= ±0.016°C) and VitalSense (0.002±0.014°C, LOA= ±0.028°C) the mean difference did not differ significantly from zero (both p-values>0.05). Furthermore, the CorTemp system demonstrated the highest mean difference between Trial 1 and Trial 2 (p=0.001), whereas the other systems had a comparable mean difference between both trials (p>0.05). The SEM was 0.058°C for CorTemp, 0.023°C for e-Celsius, 0.006°C for myTemp and 0.010°C for the VitalSense system. An excellent agreement between Trial 1 and Trial 2 was found for all four capsule systems (ICC=1.00, p<0.05).

Inertia. Inertia characteristics are summarized in Table 4. The raw data revealed that the CorTemp system had a significant lower time delay to reach p50 (9±5 seconds) and p90 (10±5 seconds) compared to the other capsule systems, whereas the VitalSense system demonstrated the slowest response (p50= 54±12 seconds, p90= 35±3 seconds; p<0.001). After correction for the systematic bias of each capsule system, the myTemp system demonstrated the lowest p50 and p90, followed by the CorTemp and e-Celsius system. The p50 and p90 remained the highest for the VitalSense system (p<0.001). Additional correction for sample frequency did not alter inertia characteristics (Table 3). Time constants of the systems response were 22 seconds for myTemp, 28 seconds for e-Celsius, 47 seconds for CorTemp and 48 seconds for VitalSense.

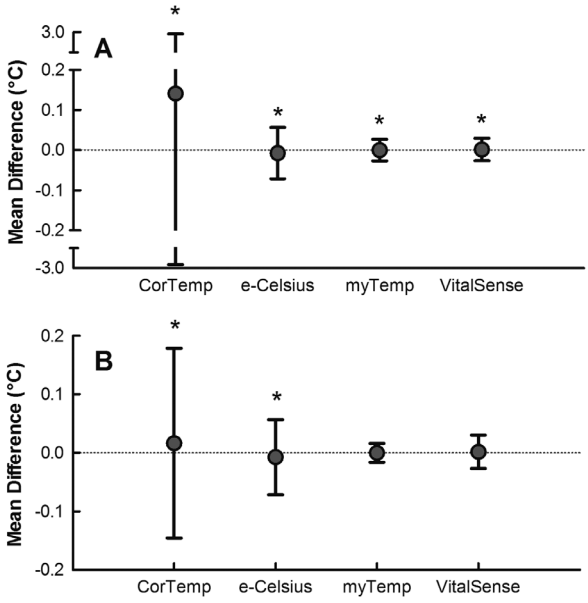


Figure 4. Raw data **(A)** and data after outlier removal **(B)** mean difference between temperatures measured during Trial 1 and Trial 2 for the capsule systems. Data were presented as mean difference ± LOA. * indicates a significant systematic

Table 4. Inertia characteristics of the four temperature capsule systems.

		CorTemp	e-Celsius	myTemp	VitalSense	p-value
Raw data	p50 (s)	9±5[b,c,d]	41±17[a,c]	23±2[a,b,d]	54±12[a,c]	**<0.001**
	p90 (s)	10±5[b,c,d]	27±9[a,d]	23±3[a,d]	35±3[a,b,c]	**<0.001**
Correction I	p50 (s)	28±8[d]	33±12[c]	22±2[b,d]	44±7[a,c]	**<0.001**
(accuracy)	p90 (s)	30±6[d]	33±11[c,d]	21±1[b,d]	45±8[a,b,c]	**<0.001**
Correction II	p50 (s)	25±4[d]	21±13[d]	19±2[d]	39±6[a,b,c]	**<0.001**
(accuracy + sample frequency)	p90 (s)	26±7[d]	21±9[d]	18±1[d]	38±9[a,b,c]	**<0.001**

Data were presented as the delay of capsule systems to reach p50 and p90 compared to the water bath. [a] represents significantly different from CorTemp, [b] different from e-Celsius, [c] different from myTemp and [d] different from VitalSense.

DISCUSSION

This is the first study to compare the validity, reliability and inertia characteristics of all commercially available ingestible telemetric temperature capsule systems. Our well controlled ex-vivo water bath study demonstrates that all temperature capsule systems, are valid and reliable to measure (water) temperature, evidenced by their small systematic biases and a low LOA and SEM after removal of outliers (CorTemp). Furthermore, we found that the CorTemp, e-Celsius and myTemp capsule system demonstrated comparable inertia characteristics, whereas the VitalSense system demonstrated a lower responsiveness to changes in water bath temperature. These findings enable researchers and clinicians to select the telemetric capsule system that best suits their goal, which can improve the safety aspect of doing exercise in a hot and cold environment.

An excellent validity and reliability of a temperature measurement technique is characterized by a 1) low systematic bias (<0.1°C), 2) narrow 95% LOA (maximal ±0.4°C), 3) high ICC (>0.80) with the reference temperature, and 4) low SEM[10, 13, 18]. We found a significant systematic bias for all four capsule systems, but the validity and reliability of every capsule system complied with reference criteria for an excellent acceptable level of agreement. Nevertheless, we observed a substantial prevalence of outliers in our raw CorTemp data (4.0%), leading to a high LOA (2.3°C) and violation of accuracy criteria (<0.1°C). Data verification and cleaning are, therefore, needed before CorTemp data can be used appropriately. Furthermore, the decreasing systematic bias with increasing temperatures suggests that the CorTemp system is mainly accurate in normothermic and hyperthermic conditions (36-44°C), but less accurate for hypothermic conditions (33-35°C). Although, the CorTemp system did not met the criteria for an excellent validity for hypothermic conditions, the systematic bias (0.1 - 0.2°C) is still physiologically acceptable. e-Celsius, myTemp and VitalSense were more constant and performed well across the whole temperature range. Furthermore, the intraclass correlation coefficient (ICC) and the standard error of measurement (SEM) were used to assess the reliability[17, 18]. An ICC of 1.00 was found for all capsule systems, whereas an ICC of >0.80 is typically considered as acceptable, with higher values respresenting a better reliability[18]. The high ICC of the four capsule systems suggests that the error variance between water bath and capsule temperature and between Trial 1 and Trial 2 are negligible compared to the normal variance of the measurement[19]. Addionally, the low SEM for all capsule systems is another indication that there is an excellent agreement between water bath and capsule temperature and between Trial 1 and Trial 2. Therefore, all capsule systems are valid and reliable methods to measure temperature after outliers have been removed.

The responsiveness of the temperature capsules was quantified by the inertia characteristics at p50 and p90. We found that the VitalSense system had the slowest response (38-39 seconds) to acute changes in temperature compared to the other systems (range: 18-26 seconds). Nevertheless, all systems demonstrated an acceptable responsiveness to changes in temperature. A previous

study reported a maximal Tc increase of 1°C per 5 minutes if no heat can be removed from the body[2]. An inertia of 18 to 39 seconds is, therefore, physiologically irrelevant. Moreover, the underestimation of Tc measured with a temperature capsule in dynamic and/or quick changing situations is marginal and hardly influences final Tc. Furthermore, the order of the results of the time constants matches the results of the p50 and p90 times corrected for sample frequency. The observed time constants are considered appropriate for the physiological signals measured.

Even though the results of our study may be promising, practical considerations must be taken into account. First, the activation of the VitalSense temperature capsules was hard and one of the capsules (10%) could not be activated at all. Anecdotal evidence from our research groups and our collaborators, confirm the infrequent non-activation problem of VitalSense capsules in other studies, whereas similar problems were occasionally experienced for CorTemp capsules. The sample frequency is also an important distinction between the capsule systems, since the sample frequency can be adjusted for CorTemp and myTemp, while it is fixed and relatively low frequent for e-Celsius and VitalSense. Furthermore, 4% of the raw CorTemp data consisted of outliers (>1°C) and another 4.4% of error measurements (0.2-1.0°C). The CorTemp system is therefore less consistent and the use of the raw data with large intervals between measurements might result in inaccurate values. Finally, the present study used capsules from a single production batch from each capsule system, which limited us to assess batch differences within capsule systems.

For human use, other aspects than the investigated accuracy, test-retest reliability and inertia, also play a role. Tc is the result of the local thermal balance affected by tissue properties and local blood flow[20]. Studies comparing different measurement location in the digestive system showed that absolute temperatures and inertia differ between locations[21, 22]. Moreover, the esophageal temperature is ~0.2°C lower during moderate intensity exercise compared to both the gastrointestinal and rectal temperature[21]. Additionally, the response time of the esophageal temperature is faster than the gastrointestinal temperature, which in turn was faster than the rectal temperature[21]. Ideally, the capsule should be located in the gastrointestinal tract and not in the stomach, which can be achieved by timely swallowing the capsule[12, 23].

In conclusion, significant but small differences were observed across telemetric temperature capsule systems. CorTemp demonstrated outliers and error measurements in 4.0% of the recorded data, while this was virtually absent in all other systems. Nevertheless, an excellent validity and test-retest reliability was found for all systems after removal of outliers. The best test-retest reliability was found for the myTemp and VitalSense system, whereas CorTemp and e-Celsius demonstrated a small, but negligible, systematic difference between Trial 1 and Trial 2. Furthermore, the VitalSense system showed the slowest response to increases in water bath temperature, while the other systems had a comparable time delay.

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Chapter 5

Pre-cooling versus Per-cooling to Improve Exercise Performance in the Heat: A Meta-Analytical Review

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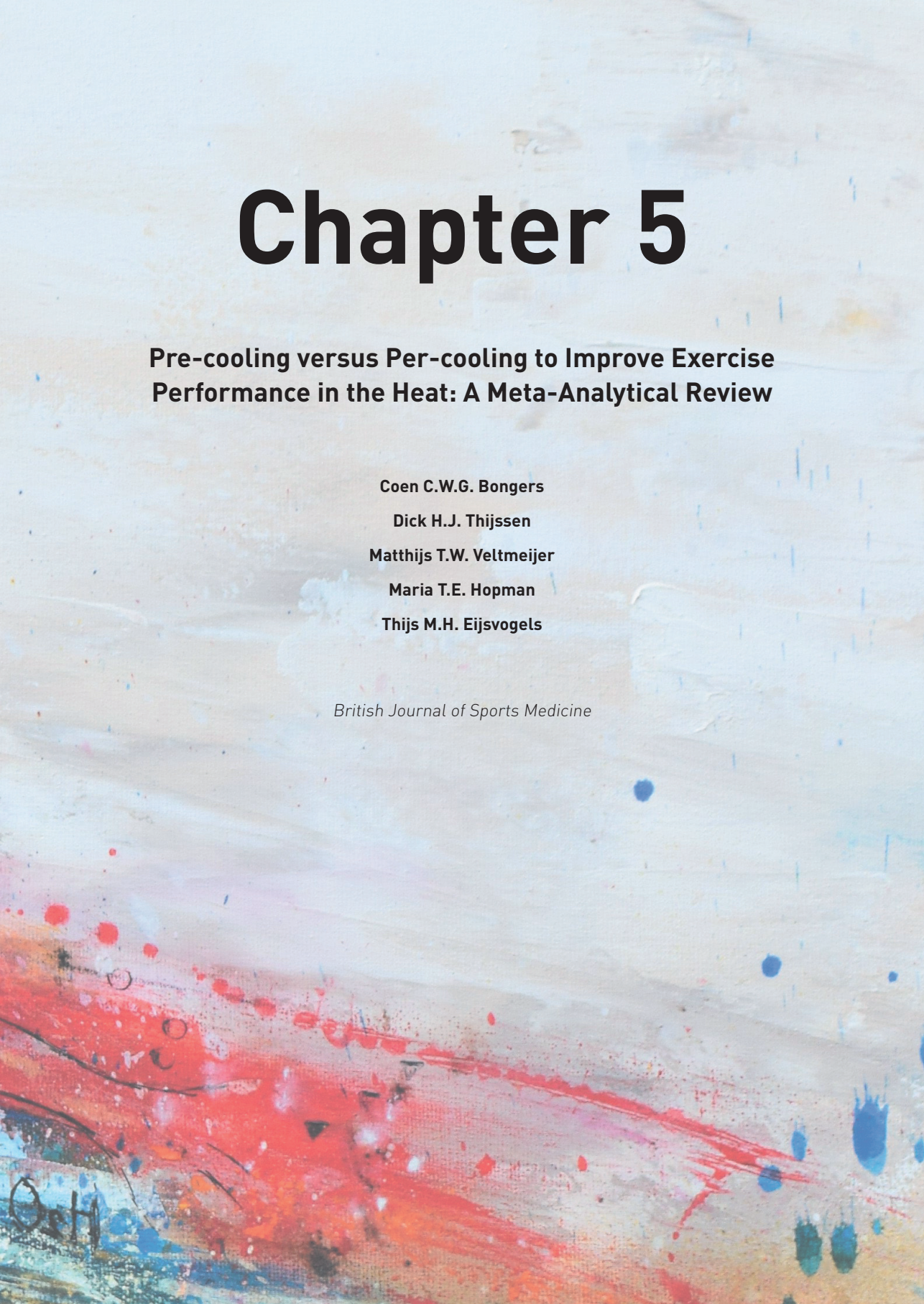
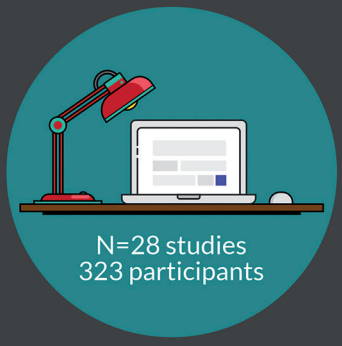


Pre-cooling and Per-cooling both improve performance in the heat: a meta-analytical review

Meta-analysis:



N=28 studies
323 participants

Inclusion criteria:

- Cooling prior to or during exercise
- Ambient temperature ≥30°C
- Male participants
- Outcome parameter associated with:

Overall effect of cooling vs control

Performance: + 6.7%

Maximum Tc: – 0.2°C

Pre-cooling (Prior to exercise)		Per-cooling (During exercise)
+ 5.7%	Performance	+ 9.9%
Mixed method cooling	Most effective cooling strategy	Ice vest cooling

Pre-cooling and per-cooling are equally effective in improving exercise performance in the heat

ABSTRACT

Exercise increases core body temperature (Tc), which is necessary to optimize physiological processes. However, excessive increase in Tc may impair performance and places subjects at risk for the development of heat-related illnesses. Cooling is an effective strategy to attenuate the increase in Tc. This meta-analysis compares the effects of cooling *before* (pre-cooling) and *during* exercise (per-cooling) on performance and physiological outcomes. A computerized literature search, citation tracking and hand search was performed up to May 2013. Twenty-eight studies met the inclusion criteria, which were trials that examined the effects of cooling strategies on exercise performance in men, whilst exercise was performed in the heat (>30°C). Twenty studies used pre-cooling, while eight studies used per-cooling. The overall effect of pre- and per-cooling interventions on exercise performance was +6.7±0.9% (effect size (ES)=0.43). We found a comparable effect (p=0.82) of pre-cooling (+5.7±1.0% (ES=0.44)) and per-cooling (+9.9±1.9% (ES=0.40)) to improve exercise performance. A lower finishing Tc was found in pre-cooling (38.9°C) compared to control condition (39.1°C, p=0.03), whilst Tc was comparable between conditions in per-cooling studies. No correlation between Tc and performance was found. We found significant differences between cooling strategies, with combination of multiple techniques being most effective for pre-cooling (P<0.01) and ice vest for per-cooling (P=0.02). Cooling can significantly improve exercise performance in the heat. We found a comparable effect size for pre-cooling and per-cooling on exercise performance, while the type of cooling technique importantly impacts the effects. Pre-cooling lowered the finishing core temperature, whereas there was no correlation between Tc and performance.

INTRODUCTION

Excessively elevated core body temperature (Tc), arising from a disbalance between heat production and heat loss during prolonged exercise, has a negative impact on physiological functions and exercise performance[1, 2]. Moreover, an elevated Tc can even lead to the development of severe heat illnesses, such as heat stroke[2]. The relevance of attenuating the increase in Tc during exercise is highlighted by the organization of future major sport events in hot and/or humid climatic conditions (e.g. Olympic Games of Rio de Janeiro 2016 and the FIFA World Cup in Brazil 2014 and Qatar 2022). Moreover, the level of performance decrement progressively increases with a rise in environmental heat stress[3]. Strategies that can prevent excessive heat storage during exercise in the heat, and consequently a reduction in exercise performance, are therefore of high interest.

Cooling can be applied prior to (*pre-cooling*) or during (*per-cooling*) exercise to attenuate the increase in Tc and improve exercise performance. Existing reviews and meta-analyses showed that pre-cooling can effectively enhance exercise performance[4-7]. A substantially lower number of studies focused on cooling strategies applied *during* exercise: per-cooling. Performance benefits of pre-cooling normally decrease after 20-25 minutes of exercise[8]. Therefore, the use of cooling techniques *during* an exercise bout, especially when involving endurance exercise, may elongate the duration of the beneficial effects of the cooling intervention on exercise performance. In addition to the larger 'window of opportunity' to cool the athlete, the level of thermal strain is higher during exercise compared to resting conditions[9]. This suggests that cooling during exercise has a large potential in clinical practice to prevent significant thermal strain and maintain exercise performance. These cooling strategies are referred to as per-cooling, derived from the Latin word *per* meaning 'during'. To date, relatively little is known about the impact of per-cooling on exercise performance, or examined the hypothesis that per-cooling is more effective than pre-cooling[10].

The purpose of this meta-analytical review is to compare the effects of pre-cooling and per-cooling on exercise performance and on relevant thermophysiological outcomes (*i.e.* core body temperature, skin temperature and heart rate) in healthy volunteers under hot climatic conditions. Furthermore, the effects of pre- and per-cooling on performance may vary between cooling techniques (cooling vests, cold water immersion, cold water ingestion, cooling packs, and mixed method cooling)[4-6, 11-13]. Better insight into these techniques is necessary to identify the 'best practice' cooling technique to improve exercise performance under hot thermal conditions. Therefore, the second aim of this study is to review current literature on this topic and determine differences between cooling techniques.

METHODS

Search strategy

We searched Pubmed and Web of Science. Ten mesh terms and keywords ('exercise', 'cooling', 'performance', 'during exercise', 'pre-cooling', 'effects', 'ice slurry ingestion', 'cooling vest', 'cold water immersion', and 'cold water ingestion') were combined by Boolean logic (AND) and the results were limited to human subjects and articles written in English. Each database was searched from their earliest available article up to May 7, 2013. We also searched in the reference list of all incoming articles.

Study selection

Selection of publications for inclusion in this meta-analysis was based on the following criteria. First, only studies applying a cooling intervention before ('pre-cooling') or during exercise ('per-cooling') and in a crossover design were selected. Moreover, only studies performed in hot ambient conditions with ambient temperatures ≥30°C were included. Secondly, only study populations comprising male adults, or studies comprising both sexes where data of male subjects was reported separately were included to avoid any potential impact of the menstrual cycle on study results. Furthermore, only studies reporting at least one outcome parameter associated with cycling or running exercise performance (*e.g.* finish time, completed distance, time to exhaustion, power output, etc.) were included in this meta-analysis. Studies that merely evaluated the effects of cooling on physiological outcomes (heart rate and blood lactate levels) were excluded. The first author was responsible for the study selection. After the selection process, all studies were discussed with two co-authors. In case of disagreement about the inclusion of a study, a voting process was used to determine if a study was included or not. Figure 1 provides a flow chart of our literature search.

Study classification

After inclusion, studies were classified into groups based on the following criteria. For our first aim, studies were classified based on the type of cooling (pre-cooling *versus* per-cooling). For our second aim, studies were classified according to their cooling strategy: 1) cooling vest (ice vests and evaporative cooling vests), 2) cold water immersion, 3) cold water ingestion and/or ice slurry ingestion, 4) cooling packs, and 5) mixed method cooling (combined application of two or more cooling techniques). Furthermore, studies that compared multiple cooling intervention trials with the same control condition, were included more than once.

Effect size assessment

For all studies that were included, standardized mean differences (effect size in Hedge's g) and 95% confidence intervals were calculated for continuous outcomes using the Cochrane Collaboration's software Review Manager 5.1.0 (Cochrane IMS, Melbourne, Australia). Statistical analyses were also performed using this software, with the significance level set at $p<0.05$. The calculations in this

program were based on the difference in outcome between the intervention and the control condition. To calculate the standard error, we needed the exact p-value (for calculation of the t-value). When the p-value was not provided, we contacted the corresponding author. However, if this information could not be provided or the author did not respond, we used p=0.049 and p=0.051 for P<0.05 and P>0.05 respectively. This progressive approach avoids an overestimation of the effect of cooling. However, as it may also cause a selection bias, we performed a sub-analysis including only studies that provided the exact p-values. Negative effects of cooling were indicated with a minus sign. Data for all single studies and weighted average values were presented as mean±SD. The interpretation of the effect size (ES) was based on the following scale: 0-0.19 = negligible effect, 0.20-0.49 = small effect, 0.50-0.79 = moderate effect and ≥0.80 = large effect[14]. The presence of publication bias was established by evaluating Begg's funnel plot asymmetry[15] and the Egger's linear regression test[16], in which p<0.05 was considered significant[17].

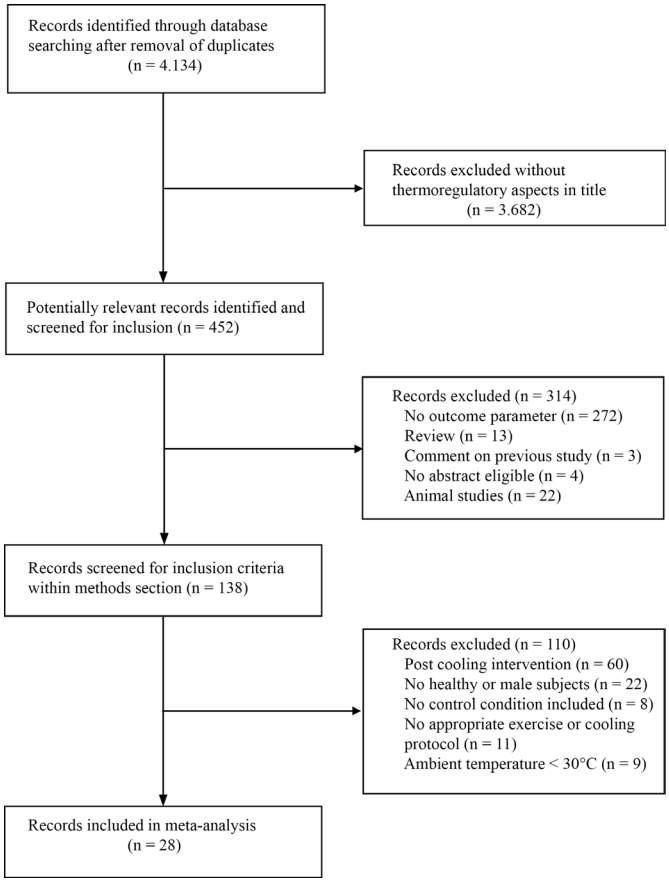


Figure 1. Overview of selection process of the included studies for this meta-analysis. N=number of studies.

Physiological parameters

We included core temperature (Tc), skin temperature (Tskin) and heart rate (HR) in this meta-analysis. Data was extracted from text, tables or figures (using GetData Graph Digitizer software v2.26). The effect of the cooling intervention was calculated by subtracting data of the cooling condition from the control condition (ΔTc, ΔTskin and ΔHR). Correlations between the change in physiological responses and the relative change in performance were calculated using SPSS 20.0 (SPSS, Chicago, USA) and the level of significance was set at $p<0.05$. A Student's paired t-test was used to examine differences in finishing Tc, Tskin and HR between the cooling and the control condition.

RESULTS

Included studies

A total of 28 manuscripts that met our inclusion criteria[11, 12, 18-43] were identified. Some of these studies compared multiple cooling interventions and were therefore included more than once, which resulted in a total of 36 studies with a total number of 323 subjects. The average sample size was 9, while the largest study was based on 20 subjects. The weighted average improvement of the cooling strategies on exercise performance in all studies was 6.7±0.9% and the weighted average ES was 0.43±0.06. A funnel plot of all studies demonstrates the presence of publication bias due to asymmetry (Figure 2). The publication bias was confirmed by a statistical significant Egger's test ($p<0.01$) and a significant Begg's funnel plot ($p=0.01$). The sub-analysis, in which the studies with exact p-values were included only, did not alter the outcomes of the original analysis. Therefore, only data from the initial analysis are provided.

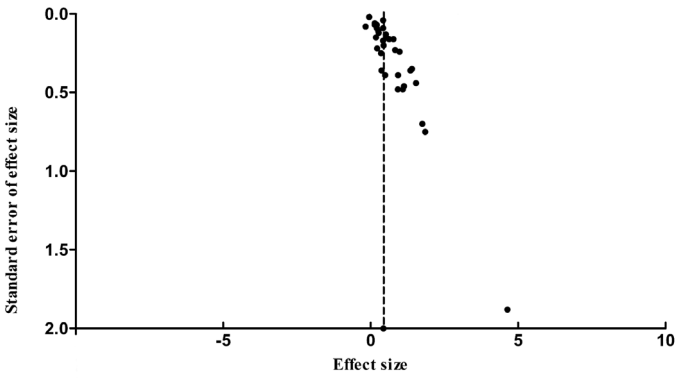


Figure 2. The Funnel plot analysis indicated a possible presence of publication bias due to the asymmetrical shape. The vertical dotted line represents the weighted average effect size of all included studies. The x-axis showed the effect size is shown and the y-axis the standard error of the effect size.

Pre-cooling *versus* per-cooling

Twenty-seven studies applied a pre-cooling intervention and nine studies applied per-cooling (Figure 3). The weighted average exercise performance improvement of pre-cooling was 5.7±0.9% (ES=0.44) and for per-cooling interventions 9.9±1.9% (ES=0.40). We found no significant difference in effect size for both types of cooling on exercise performance in the heat (p=0.82).

Effects on physiological parameters

Table 1 shows an overview of the (change in) physiological parameters during the control and cooling condition. We found a significantly lower finishing Tc in the pre-cooling (38.9°C) condition compared to control (39.1°C, p=0.03), whilst Tc was comparable for the per-cooling studies. Tskin and HR did not differ between the cooling and control condition for both pre-cooling and per-cooling (all p-values>0.05). Furthermore, no correlations were found between measures of performance and ΔTc, ΔTskin and ΔHR for pre-cooling, per-cooling and the pooled set of cooling studies (all p-values>0.05).

Different cooling techniques

Pre-cooling. We found that the effect of the different cooling strategies on exercise performance significantly differed across pre-cooling techniques (p<0.001). Mixed method cooling (+7.3%, ES=0.72, Figure 3) demonstrated a significantly larger effect size (p<0.01) compared to cold water immersion (+6.5%, ES=0.49), cold water/ice slurry ingestion (+6.3%, ES= 0.40), cooling packs (+4.3%, ES= 0.40), and cooling vests (+3.4%, ES= 0.19) (Table 2).

Per-cooling. For per-cooling studies, three different cooling techniques were identified; ice vest, cold water ingestion and cooling packs (Table 2). We found a significant difference in effect size between the 3 per-cooling techniques in our meta-analysis (p=0.01). Wearing an ice vest during exercise (+21.5%, ES= 4.64) was significantly more effective in improving exercise performance compared to cold water ingestion (+11%, ES= 1.75) and cooling packs (+8.4%, ES= 0.39) (p=0.02, Table 2).

DISCUSSION

The purpose of this meta-analysis was to 1) compare the effects of pre-cooling *versus* per-cooling on exercise performance and thermophysiological responses in the heat, and 2) to identify the most effective cooling technique for improvement in exercise performance. Reviewing and analyzing data of the existing studies indicates that cooling significantly improves exercise performance, whilst the effect of cooling was similarly present between pre-cooling and per-cooling. Secondly, thermophysiological (such as core and skin temperature and heart rate) outcomes did not change in response to both pre-cooling and per-cooling, whilst no correlation was present between the change in thermophysiological measures and

exercise performance. Thirdly, we found significant differences between pre-cooling techniques to improve exercise performance, with the use of a mixed method of cooling being the most effective. Such an effect between different techniques was also observed for per-cooling, with an ice vest being the most effective strategy. Taken together, cooling prior to or during exercise in the heat improves exercise performance with evidence supporting a superior effect of mixed methods for pre-cooling and ice vests for per-cooling on performance levels in athletes, whilst these performance effects are unlikely related to a lower skin or core body temperature.

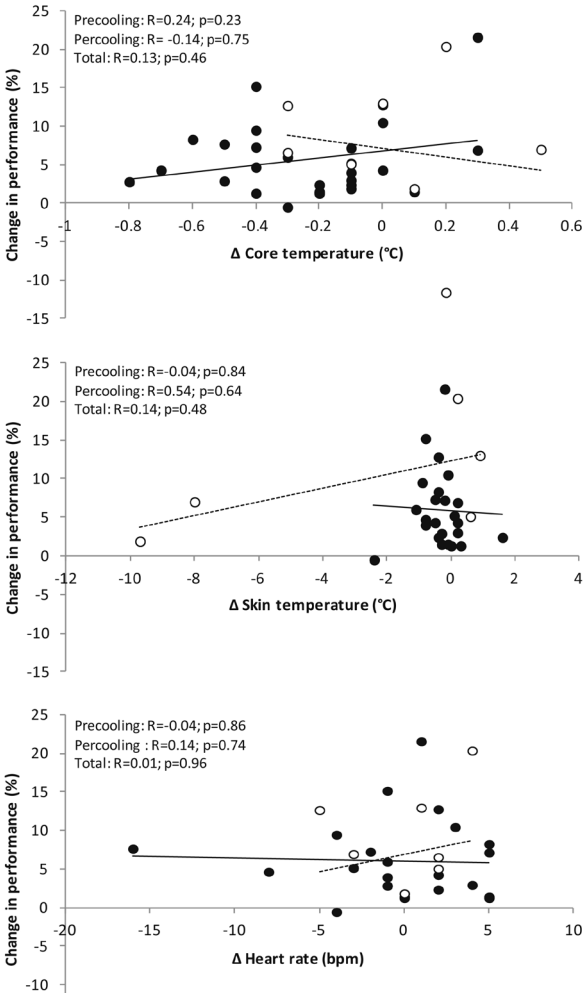


Figure 4. Correlations between change in exercise performance (%) and change in core temperature (ΔTc), skin temperature (ΔTskin) and heart rate (ΔHR) for both pre-cooling (●) and per-cooling (○). Pearson's correlation coefficient, significance assumed at p<0.05. Delta (Δ) = cooling – control.

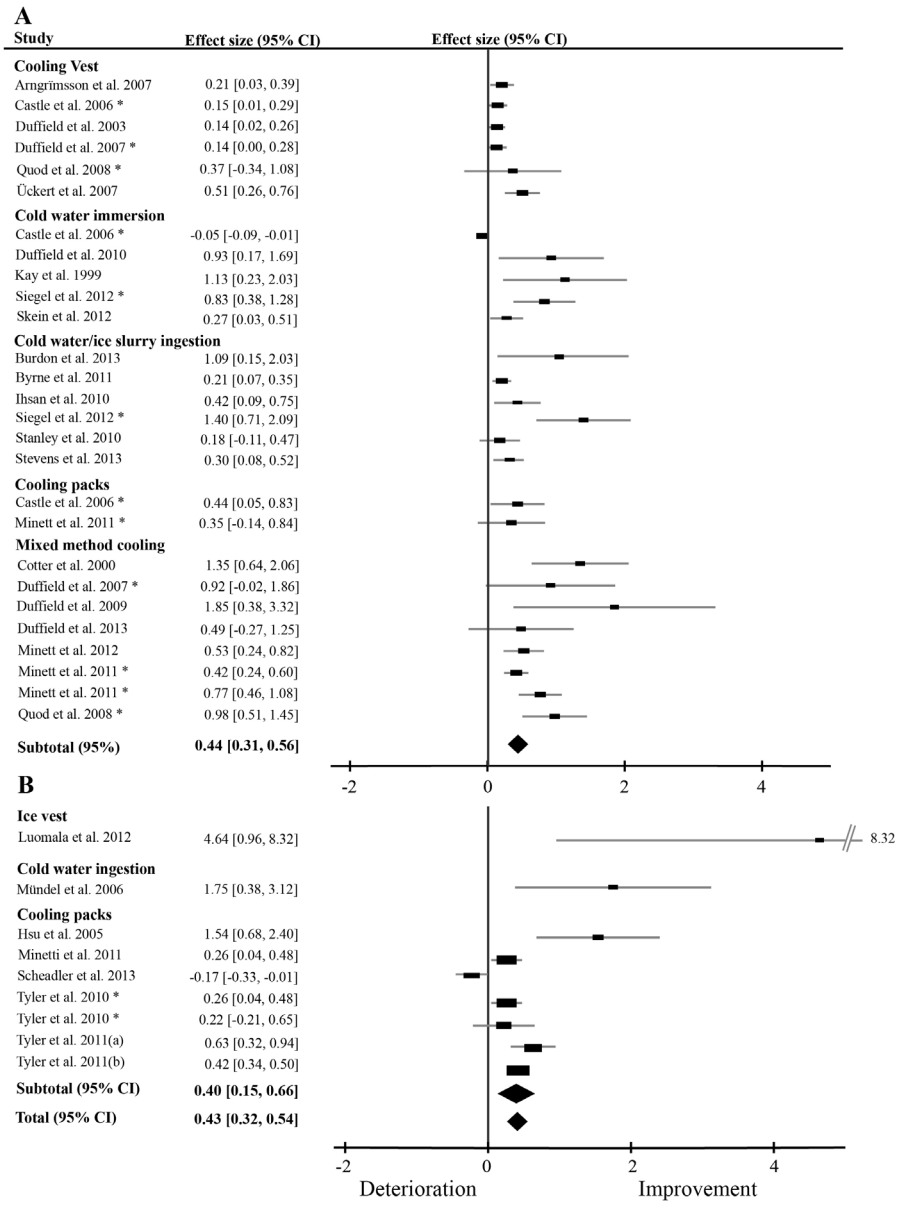


Figure 3. Forest plot summarizing the effects of different cooling techniques on exercise performance for the pre-cooling **(A)** and the per-cooling studies **(B)**. The magnitude of the effect size indicates: 0-0.19 = negligible effect, 0.20-0.49 = small effect, 0.50-0.79 = moderate effect and ≥0.80 = large effect[14]. The black rectangles represented the weighted effect size and the grey lines are the 95% confidence intervals. The size of the rectangles indicated the weight of the study, which is calculated separately for the precooling and per-cooling studies.* Studies that used multiple cooling intervention trials were included more than once.

Table 1. Individual study data regarding the physiological responses, in which Δ were calculated as cooling minus control condition.

Pre-cooling		Tc max control	Tc max cooling	Δ Tc max	Tskin max control	Tskin max cooling	Δ Tskin max	HR max control	HR max cooling	Δ HR max	Performance (%)
Cooling packs	Castle et al. 2006c	39.1	38.4	-0.7	36.9	36.4	-0.5	179	181	2	4.3
	Minett et al. 2011a	39.1	39.1	0	34.0	34.2	0.2	173	175	2	4.3
	Weighted average	39.1	38.8	-0.4	35.5	35.3	-0.1	176	178	2	4.3
Cooling vests	Arngrimsson et al. 2004	39.8	39.6	-0.2	34.2	34.5	0.3	195	195	0	1.3
	Castle et al. 2006a	39.1	38.9	-0.2	36.9	36.6	-0.3	179	184	5	1.5
	Duffield et al 2003	38.8	38.7	-0.1	34.0	33.6	-0.4	N.A	N.A	N.A	2.4
	Duffield et al. 2007a	39.6	39.2	-0.4	34.4	34.4	0	182	187	5	1.3
	Quod et al. 2008a	39.6	39.7	0.1	34.6	34.5	-0.1	193	193	0	1.5
	Ückert et al. 2007	38.8	38.4	-0.4	35.6	35.1	-0.5	192	190	-2	7.3
	Weighted average	39.3	39.1	-0.2	35.0	34.8	-0.2	188	190	2	3.4
Cold water ingestion	Burdon et al. 2013	38.7	38.7	0.0	33.4	33.3	-0.1	165	168	3	10.5
	Byrne et al. 2011	38.6	38.1	-0.5	35.4	35.1	-0.3	190	189	-1	2.9
	Ihsan et al. 2010	38.8	39.1	0.3	35.6	35.8	0.2	N.A	N.A	N.A	6.9
	Siegel et al. 2012a	39.5	39.8	0.3	35.7	35.5	-0.2	188	189	1	12.8
	Stanley et al. 2010	39.1	39.0	-0.1	N.A	N.A	N.A	191	191	0	1.9
	Stevens et al. 2013	39.0	38.2	-0.8	N.A	N.A	N.A	N.A	N.A	N.A	2.8
	Weighted average	39.0	38.8	-0.1	35.0	34.9	-0.1	184	184	1	6.3
Mixed method cooling	Cotter et al. 2000	38.9	38.5	-0.4	35.9	35.1	-0.8	178	177	-1	15.2
	Duffield et al. 2007b	39.6	39.0	-0.6	34.4	34.0	-0.4	182	187	5	8.3
	Duffield et al. 2009	39.3	38.8	-0.5	N.A	N.A	N.A	162	146	-16	7.7
	Duffield et al. 2013	39.0	38.9	-0.1	34.6	34.8	0.2	182	186	4	3.0
	Minett et al. 2011b	39.1	39.0	-0.1	34.0	34.1	0.1	173	170	-3	5.2
	Minett et al. 2011c	39.1	38.7	-0.4	34.0	33.1	-0.9	173	169	-4	9.5
	Minett et al. 2012	39.1	38.7	-0.4	33.9	33.1	-0.8	178	170	-8	4.7
	Quod et al. 2008b	39.6	39.5	-0.1	34.6	33.8	-0.8	193	192	-1	4.0
	Weighted average	39.1	38.9	-0.3	34.5	34.0	-0.5	178	175	-3	7.3
Cold water immersion	Castle et al. 2006b	39.1	38.8	-0.3	36.9	34.5	-2.4	179	175	-4	-0.5
	Duffield et al. 2010	39.0	38.9	-0.1	35.7	35.5	-0.2	178	183	5	7.2

	Kay et al. 1999	38.8	38.5	-0.3	34.7	33.6	-1.1	178	177	-1	6.0
	Siegel et al. 2012b	39.5	39.5	0	35.7	35.3	-0.4	188	190	2	21.6
	Skein et al. 2012	38.9	38.7	-0.2	31.5	33.1	1.6	180	182	2	2.4
	Weighted average	39.1	38.9	-0.2	34.9	34.4	-0.5	181	181	1	6.5
Cooling packs	Hsu et al. 2005	38.4	38.1	-0.3	N.A	N.A	N.A	159	161	2	6.6
	Minetti et al. 2011	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	5.4
	Scheadler et al. 2013	39.2	39.4	0.2	N.A	N.A	N.A	178	178	0	-11.6
	Tyler et al. 2010a	39.3	39.1	-0.1	35.0	35.6	0.6	186	188	2	5.1
	Tyler et al. 2010b	38.3	38.4	0.1	35.8	26.1	-9.7	187	187	0	1.9
	Tyler et al. 2011a	39.2	39.7	0.5	35.6	27.6	-8	181	178	-3	7.0
	Tyler et al. 2011b	38.9	38.9	0	34.4	35.3	0.9	185	186	1	13.0
	Average	38.9	38.9	0.1	35.2	31.2	-4.1	179	180	0	3.9
Cooling vest	Luomala et al. 2012	38.9	39.1	0.2	34.5	34.7	0.2	174	178	4	20.4
	Average	38.9	39.1	0.2	34.5	34.7	0.2	174	178	4	20.4
Cold water ingestion	Mündel et al. 2006	38.7	38.4	-0.3	N.A	N.A	N.A	170	165	-5	12.7
	Average	38.7	38.4	-0.3	N.A	N.A	N.A	170	165	-5	12.7
Total per-cooling	**Average**	**38.9**	**38.9**	**0.0**	**35.1**	**31.9**	**-3.2**	**178**	**178**	**0**	**7.0**
	Students T-test	**0.91**			**0.16**			**0.98**			
Total all studies	**Average**	**39.1**	**38.9**	**-0.2**	**34.9**	**34.1**	**-0.8**	**180**	**180**	**0**	**5.6**
	Students T-test	**0.08**			**0.08**			**0.97**			

Tc = core body temperature; Tskin = skin temperature; HR = heart rate; N.A = not available; max = at the end of the exercise protocol

Table 2. Overview of subtotal effect sizes ± 95% CI of different cooling techniques for the pre-cooling and per-cooling interventions.

	Number of studies	Pre-cooling	Number of studies	Per-cooling
Cooling vest	6	0.19 (0.10-0.28)	1	4.64 (0.96-8.32)
Cold water immersion	5	0.49 (0.09-0.90)	-	Not available
Cold water ingestion	6	0.40 (0.17-0.62)	1	1.75 (0.38-3.12)
Cooling packs	2	0.40 (0.10-0.71)	7	0.34 (0.09-0.58)
Mixed method cooling	8	0.72 (0.49-0.96)	-	Not available
Average effect size	27	0.44 (0.31-0.56)	9	0.40 (0.15-0.66)

Our analysis summarizes and demonstrates a significant effect of cooling interventions on exercise performance in healthy athletes under demanding thermal conditions[1, 7, 44]. We extend the current knowledge by the observation that the impact of pre-cooling and per-cooling on exercise performance is comparable. It is important to take note of the significant publication bias, which is demonstrated in the Funnel plot (Figure 2), suggesting that negative studies may not have been published. Although this could implicate an overestimation of the overall effect of cooling, there is still abundant evidence that cooling effectively improves exercise performance when exercise is performed in the heat. The application of pre- and per-cooling are therefore both recommended to improve exercise performance while exercising in hot ambient conditions.

Although our statistical analysis does not support a difference in effect size between pre- and per-cooling (ES = 0.44 *versus* 0.40), the variation in performance enhancement between pre-cooling (+5.7%) and per-cooling (+9.9%) is large. It is believed that both cooling strategies achieve their effects through comparable underlying mechanisms. It is known that exercise leads to a significant level of thermal strain due to a large increase in heat production in the exercising muscles. Maintaining an adequate heat balance requires a significant amount of energy for heat dissipating mechanisms, such as (skin) vasodilation and sweating responses[9, 45]. Per-cooling contributes to a higher heat storage capacity, a more efficient heat loss and may attenuate the increase in core body temperature. The attenuated increase in Tc, may prevent a decrease in exercise performance. The purpose of pre-cooling is to lower Tc before starting the exercise, leading to an increase in heat storage capacity during exercise. It is hypothesized that the larger heat buffer, induced by pre-cooling, enables the body to perform more work prior reaching a critical limit for Tc[13]. This suggests that pre- and per-cooling both enhance exercise performance. Accordingly, we hypothesize that a combination of pre-cooling and per-cooling may be more effective in improving exercise performance than a single cooling strategy only. To date, only one pilot study (n=9) examined this hypothesis and showed that combined pre- and per-cooling is superior in improving exercise performance compared to pre- or per-cooling alone[46]. Future studies may be aimed to further explore the combined effect of pre- and per-cooling on exercise performance.

One important question that this meta-analysis tried to answer is whether the impact of cooling strategies can be explained through its effects on thermophysiological factors. Pre-cooling resulted in a significantly lower finishing Tc in the cooling compared to control condition, whereas this finding was absent in per-cooling studies. Presumably, per-cooling attenuated the increase in Tc and thus increase the heat storage capacity. For this reason, athletes were able to produce more heat before terminating exercise or lowering exercise intensity, which results in performance enhancements[10, 33]. Likewise, we did not find correlations between the change in physiological parameters and the improvement of performance (Figure 4). These findings suggest that a lower Tc at the end of exercise does not necessarily improve exercise performance in the heat. More likely, the cooling interventions resulted in a reduction of the rise in physiological parameters, which enabled athletes to exercise at a higher absolute amount of work resulting in an improved performance but a comparable finishing Tc, Tskin and HR[5].

None of the included studies reported any thermoregulatory problems or heat related illnesses amongst their subjects. This may imply that our body applies internal protection mechanisms to avoid reaching a critical high temperature. There are 2 common hypotheses that may explain this thermal behavior. Firstly, as Tc becomes elevated, exercise will be terminated once critically high internal temperatures are attained, which is a safeguard that limits the potential development of dangerous heat illness[5, 6]. Secondly, the rate of heat gain is detected by our body, which could anticipatorily adjust the work rate to ensure that the exercise task can be completed within the homeostatic limits of the body[5, 47]. As this meta-analysis included merely information about peak Tc, we could not test which hypothesis was adopted by athletes while performing exercise in the heat. Future studies that compare the threshold- with the anticipatory-theory are recommended, so that appropriate cooling techniques can be selected accordingly.

This meta-analysis demonstrated a significant impact of the type of cooling strategy when performing pre-cooling to enhance exercise performance. Our analysis revealed that a combination of techniques (*i.e.* 'mixed method pre-cooling') had a significantly larger effect than individual cooling techniques (cold water/ice slurry ingestion, cooling vests, cooling packs, or cold water immersion alone). This observation is reinforced by a study which examined three pre-cooling strategies; 1) cooling pack, 2) cooling pack + cold water immersion, and 3) cooling pack + cold water immersion + ice vest[27]. Whilst no effect was found for the cooling pack, both mixed method cooling trials effectively improved exercise performance[27]. The higher cooling capacity in the mixed method cooling compared to individual cooling strategies likely contributes to this finding. Especially mixed techniques with an 'aggressive' approach and affecting a large body surface seem to contribute to a larger effect on exercise performance. The law of enthalpy of fusion states that ice possesses significantly greater capacity to absorb heat than liquid water[6, 48, 49]. Accordingly, more aggressive cooling techniques, typically depending on ice or substances with a temperature below zero, demonstrate a larger effect

on changing core body temperature and/or exercise performance. In addition, previous data supports the idea that whole body cooling is more effective than cooling of a part of the body only[27]. Indeed, despite the use of a relatively mild stimulus (*i.e.* 14-24°C), full-body water immersion significantly improved exercise performance[18, 21, 25, 31]. The large cooling surface may importantly contribute to the prolonged suppression of increased physiological and thermal loads[22, 50], and thus improve exercise performance. Taken together, a combination of pre-cooling techniques, preferably 'aggressive' cooling and interventions that cover a substantial part of the athlete´s body, represent the current 'best practice' model for pre-cooling to improve exercise performance.

Also for the per-cooling strategies, our meta-analysis revealed a significant impact of the type of cooling. Our analyses indicate that wearing an ice-vest during exercise has a significantly larger effect than other per-cooling techniques (cold water ingestion and cooling packs). Interestingly, the ice vests represent an aggressive cooling strategy that impacts upon a relatively large body surface. This provides further support that also during per-cooling, strategies with an aggressive nature that aim at a relatively large body surface area are the most effective cooling strategies. An important limitation is that we only included a single study on the impact of an ice vest, which coincidently reported a remarkably large effect size. Nonetheless, the similarities between the type of most effective cooling strategies for pre- and per-cooling is striking. We strongly support future studies to confirm this finding using well-controlled, within-subjects designs, but also to improve our understanding why and how these aggressive types of cooling are more successful.

Practical recommendations

Our meta-analysis combined the results of 323 subjects in 28 peer-reviewed publications and demonstrated the practical value of cooling strategies to improve exercise performance in the heat. More importantly, we showed that pre- and per-cooling are equally effective in improving exercise performance in the heat. Therefore, a combination of pre- and per-cooling may be superior compared to a single strategy alone. Moreover, we revealed that a combination of cooling techniques (for pre-cooling) or ice vests (for per-cooling) results in the largest effect size on exercise performance, possibly due to the aggressive approach and impact on a relatively large body surface. Based on our novel observations, we recommend future studies to investigate the practical performance and effect size of combining pre- and per-cooling strategies on exercise performance, preferably using aggressive types of strategies. Such joint efforts can further improve exercise performance in the heat, while it also may contribute to a reduction in heat-related illnesses in athletes.

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Chapter 6

Cooling interventions for athletes: an overview of effectiveness, physiological mechanisms, and practical considerations

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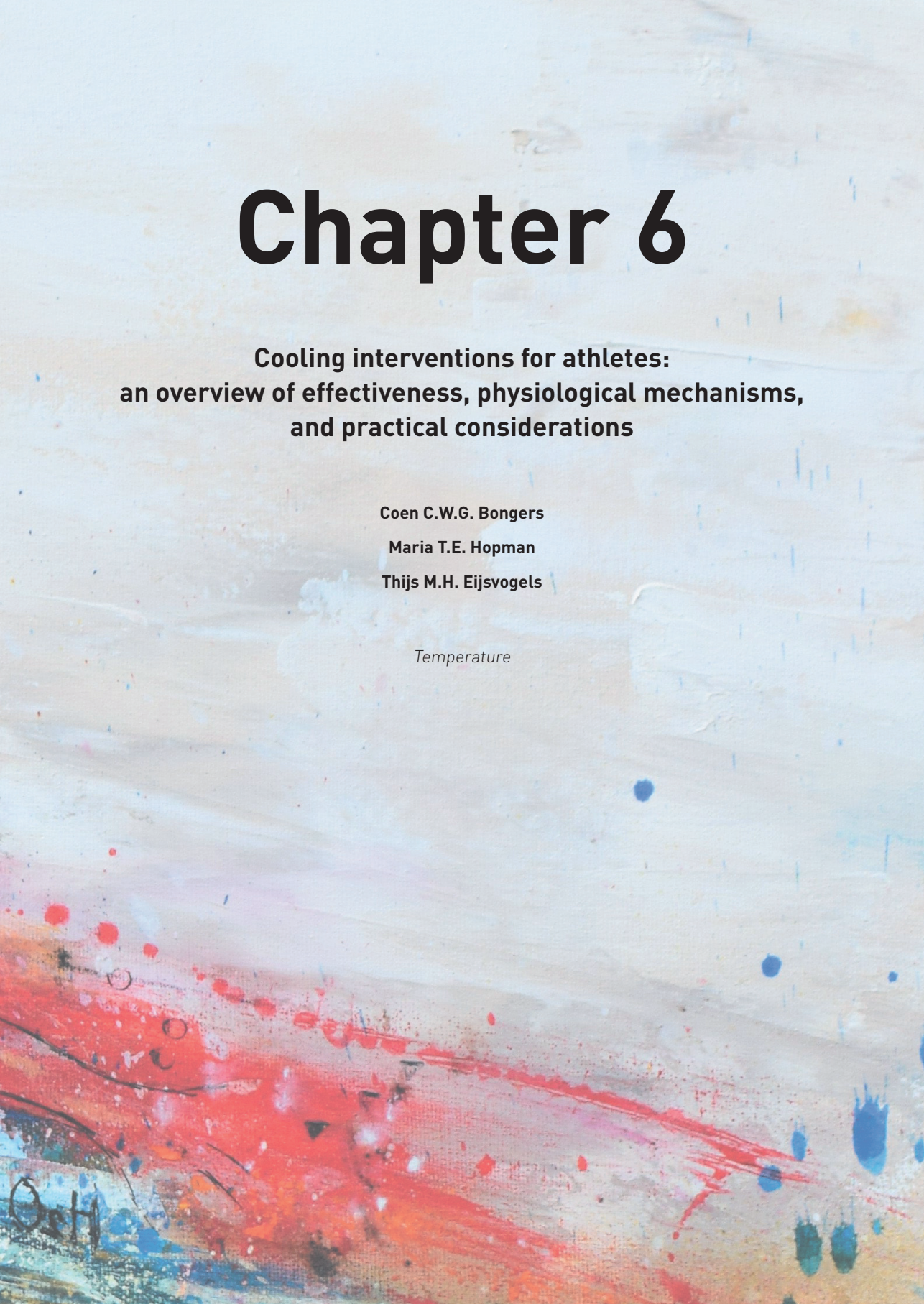
Temperature

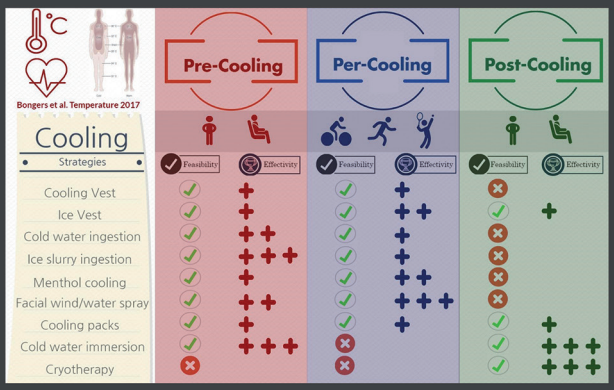


Cooling interventions for athletes: an overview of effectiveness, physiological mechanisms and practical considerations

An optimal strategy includes a vigorous cooling technique that covers a major part of the body in hot and humid conditions

Results

Practical considerations



The balance between efficiency and feasibility is important for athletes to establish their preferred cooling strategy

Proposed mechanisms

Reduces thermal strain

Reduces cardiovascular strain

Reduces metabolic strain

Anti-inflammatory response & enhance muscle recovery

Reduces perceptual strain

Any opportunity to reduce thermal strain prior to, during and/or after exercise improves performance and recovery

ABSTRACT

Exercise-induced increases in core body temperature could negatively impact performance and may lead to development of heat-related illnesses. The use of cooling techniques prior (pre-cooling), during (per-cooling) or directly after (post-cooling) exercise may limit the increase in core body temperature and therefore improve exercise performance. The aim of the present review is to provide a comprehensive overview of current scientific knowledge in the field of pre-cooling, per-cooling and post-cooling. Based on existing studies, we will discuss 1) the effectiveness of cooling interventions, 2) the underlying physiological mechanisms and 3) practical considerations regarding the use of different cooling techniques. Furthermore, we tried to identify the optimal cooling technique and compared whether cooling-induced performance benefits are different between cool, moderate and hot ambient conditions. This article provides researchers, physicians, athletes and coaches with important information regarding the implementation of cooling techniques to maintain exercise performance and to successfully compete in thermally stressful conditions.

INTRODUCTION

Human core body temperature (Tc) is regulated to ensure normal body function, while any increase of Tc above its normal range (set-point) is defined as hyperthermia[1-3]. During exercise only ~20-30% of the produced energy is converted to mechanical work, whereas ~70-80% of the energy is released as heat[4, 5]. The exercise-induced increase in heat production typically exceeds the heat loss capacity and results in Tc elevation[6, 7]. Previous studies showed significant increases in Tc in athletes exercising in cold[8], warm[9], and humid[10] environmental conditions. There is evidence of exercise-induced fatigue beyond a Tc threshold of >40°C[11] and a Tc >40.5°C may lead to the development of heat-related illnesses such as heat exhaustion, heat injury and heat stroke[12, 13]. In addition to the exercise-induced elevations in Tc, prolonged exercise also increases skin temperature[14]. A combination of an increased core- and skin temperature, resulting in a lower core-to-skin gradient, is associated with a decreased exercise performance[14-16]. Strategies to reduce the thermal strain prior to, during and directly after exercise are therefore of great importance.

Cooling interventions could increase heat storage capacity (pre-cooling), attenuate the exercise-induced increase in Tc (per-cooling) and accelerate recovery following intense exercise (post-cooling). In the past decade, several reviews and meta-analysis have been published with respect to cooling and exercise. Early reviews were primarily focused on the effects of pre-cooling on thermoregulation and exercise performance[17-21]. More recently, several overviews were published on the benefits of per-cooling and the differences between pre- and per-cooling[22-25]. However, an important limitation of previous work is that a comprehensive summary of the potential benefits of all cooling modalities for different types of sports performing exercise under different environmental conditions is missing. Furthermore, additional insight in the underlying mechanisms that are responsible for the pre-, per-, and post-cooling benefits is needed.

Therefore, the aim of the present review is to provide a comprehensive overview of current scientific knowledge in the field of pre-cooling, per-cooling and post-cooling. Based on existing studies, we will discuss 1) the effectiveness of cooling interventions on improving exercise performance, 2) the underlying physiological mechanisms and 3) practical considerations regarding the use of different cooling techniques. This article provides researchers, physicians, athletes and coaches with important information regarding the implementation of cooling techniques to maintain exercise performance and to successfully compete in thermally stressful conditions.

METHODOLOGICAL CONSIDERATIONS

The use of cooling techniques prior to, during or directly after exercise is widely described in literature, whereas large differences in study setup were found across studies. The study protocols differ with respect to cooling technique (*i.e.* cooling vests, cold water immersion, ice slurry ingestion, cooling packs, menthol cooling, facial water spray), exercise protocol (time trial, total distance covered, time to exhaustion, fixed exercise protocols), types of exercise (endurance *versus* strength) and ambient conditions (temperature, humidity). Furthermore, the sample size and study population (age, sex, level of fitness) also differed substantially across studies. This lack of standardization makes a direct comparision between studies difficult. A standardized effect size (ES) can be calculated to compare studies with different study protocols[26]. In the present review, we decided to present relative changes as well as effect sizes to demonstrate the effect of cooling on exercise performance.

In addition to the methodological differences among studies, publication bias could also influence overall outcomes. Two previous meta-analyses demonstrated a potential publication bias[22, 24], whereas other reviews did not report a potential publication bias[21, 27]. This descripancy suggests that publication bias might be present, which may implicate an overestimation of the overall effect of cooling.

PRE-COOLING

Pre-cooling can be described as the rapid removal of heat from the body prior to exercise to create a larger heat storage capacity[28]. An overview of used cooling techniques is shown in Table 1. Many pre-cooling techniques were proven to be effective, ranging from whole body pre-cooling such as cold water immersion[20, 29] and cold air exposure[30-32] to local cooling using cooling vests[33, 34] or cooling packs[35, 36], or internal cooling strategies such as the ingesting of cold water or ice slurry[20, 37, 38]. Furthermore, a combination of these cooling techniques (*i.e.* mixed method cooling), is often used to obtain a greater cooling power and larger reduction in Tc[34, 36, 39].

The effects of different pre-cooling techniques on exercise performance were examined within various ambient conditions and using different exercise protocols (i.e. endurance exercise *versus* (intermittent) sprint exercise). We previously demonstrated in a meta-analysis that pre-cooling improves exercise performance in the heat (ambient temperature >30°C) with 5.7±0.9% (ES=0.44)[22]. Mixed method cooling appeared to be the most effective strategy to enhance exercise performance, followed by cold water immersion, cold water/ice slurry ingestion, cooling packs and cooling vests (Figure 1)[22]. These findings suggest that vigorous cooling of a large surface of the body is more effective than local body and/or less powerful cooling techniques to improve exercise performance.

Table 1. Overview of the different cooling techniques

Cooling technique	Timing of Cooling	Intervention temperature (°C)	Advantages of cooling technique	Disadvantages and Practical considerations
Cooling vest	Precooling Per-cooling	10-20 °C	- Light weight - Easily applicable in field-based settings - Covers a large part of the body	- Less aggressive - Quick decrease in cooling power
Ice vest	Precooling Per-cooling Post-cooling	< 0 °C	- Aggressive cooling technique - Covers a large part of the body	- Heavy weight - Difficult to use in field-based settings
Cold water ingestion	Precooling Per-cooling	1-5°C	- Direct effect on core body temperature - Easily applicable in field-based settings	- Covers a small part of the body - Less aggressive
Ice slurry ingestion	Precooling Per-cooling	< 0 °C	- Direct effect on core body temperature - Easily applicable in field-based settings	- Covers a small part of the body - Potential gastrointestinal discomfort
Menthol cooling	Precooling Per-cooling	Not applicable	- Easily applicable in field-based settings	- Best way of application is not yet known
Facial wind/ water spray	Precooling Per-cooling	Wind and water temperature 10-20 °C	- Covers a large part of the body	- Difficult to use in field-based settings - No direct contact with the skin
Cooling packs	Precooling Per-cooling Post-cooling	<0 °C	- Aggressive cooling technique - Easily applicable in field-based settings	- Covers a small part of the body - Can restrict movement and improve air resistance - Heavy weight, less suitable for per-cooling
Cold water immersion	Precooling Post-cooling	10-25 °C	- Covers a large part of the body - Direct contact with the skin	- Not suitable in field-based settings
Cryotherapy	Precooling Post-cooling	< -100 °C	- Covers a large part of the body - Aggressive cooling technique	- No direct contact with the skin - Expensive

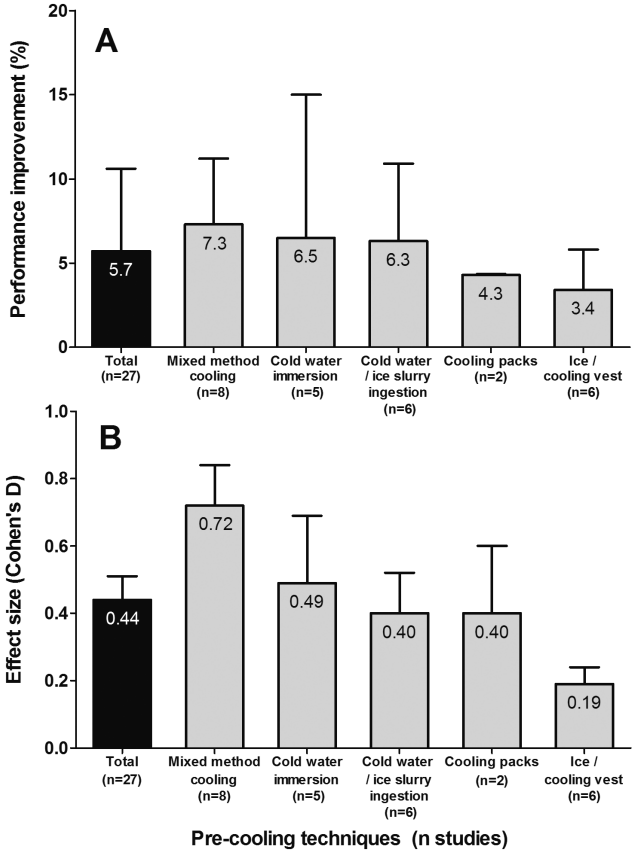


Figure 1. An overview of the average performance improvement (%) **(A)** and effect size **(B)** of pre-cooling (black bar) and the beneficial effects of different precooling strategies (grey bars). Data are presented as mean ± standard deviation. The figure is adapted from our previous meta-analysis[22].

Ambient conditions could also impact the performance benefits associated with pre-cooling. Although most pre-cooling studies were performed in simulated heat (>30°C), professional and recreational athletes will not solely practice and compete in hot ambient temperatures, but also in cool and moderate environmental conditions. Ninety minutes of cold air (0-18°C) exposure prior to exercise in moderate ambient conditions (18°C) resulted in an increased time to exhaustion[32] and an increased 1 hour work rate (172 W *versus* 161 W for cooling and control respectively)[30]. In contrast, upper body pre-cooling using an ice vest did not improve intermittent sprint exercise in a moderately warm environment (22°C dry bulb temperature, 40% relative humidity)[40]. Moreover, a decrease in exercise performance was found after 30 min of exposure to cold air (5°C) prior to 30 minutes of cycling at 50% of VO_2 max in a cold ambient temperature (5°C)[41]. The association between ambient temperature and pre-cooling

induced performance benefits was reinforced in a meta-analysis which found greater effects with increasing ambient temperatures[21].

The performance benefits of pre-cooling were confirmed by another meta-analysis[24], as pre-cooling significantly improved intermittent sprint exercise and endurance exercise performance. However, pre-cooling deteriorated single sprint performance[24]. The different impact of pre-cooling on single *versus* intermittent sprint exercise may be explained by the longer exercise duration (45-70 sec *versus* 40-80 min) and thus higher thermal stress in the intermittent sprint exercise protocols (*i.e.* soccer, field hockey, tennis and volleyball). Sprint exercise is mainly influenced by muscle temperature and anaerobic metabolism, rather than thermoregulatory factors[24, 42]. Cooled muscles have a decreased voluntary power output and might have a reduced anaerobic metabolism during sprint exercise[43, 44]. In contrast, endurance exercise includes performance of prolonged activities on a moderate to high intensity, which results in a greater thermoregulatory burden than sprint exercise. The benefits of pre-cooling are therefore larger for endurance athletes than for (intermittent) sprint athletes[24].

Taken together, the effects of pre-cooling greatly depend on the cooling strategy, exercise setting and ambient conditions. The optimal pre-cooling strategy to improve exercise performance includes a vigorous cooling technique that covers a major part of the body and is used during endurance exercise protocols in hot and humid environmental conditions.

PER-COOLING

More recently, the use of cooling techniques during exercise became of greater interest. The beneficial effects of pre-cooling normally attenuate after 20-25 minutes of exericse[45]. Therefore, cooling athletes during exercise may extend the duration of the performance benefits of a cooling intervention. Additionally, the thermal strain during exercise is much higher compared to resting or warming-up condions[6], which suggests that per-cooling should have a larger potential benefit on thermoregulation and exercise performance compared to pre-cooling. We defined per-cooling as any opportunity to reduce thermal stress during an exercise performance trial. Due to practical feasibility and sporting regulations, less cooling techniques can be applied during exercise compared to pre-cooling. Consequently, the effects of per-cooling were investigated using cooling packs[46-48], cooling vests[49, 50], cold water/ice slurry ingestion[37, 51], facial wind or water spray cooling[52, 53] and menthol cooling[54-56]. Interestingly, menthol cooling could be applied as a mouth rinse, a gel on the face or as a spray on the clothing of the athlete.

Four reviews were published with respect to per-cooling and exercise performance[22-25], of which three conducted a meta-analysis. Per-cooling literature predominantly demonstrated

improvements in exercise performance, as 15 out of 21 studies found a positive effect. In our meta-analyses, we concluded that ice vest cooling appeared to be the most effective method followed by cold water ingestion and cooling packs[22], whereas other reviews did not define the most effective per-cooling technique[23, 24]. The extrapolation of these findings were however limited, as only a single study for ice vest cooling and cold water ingestion was included in the initial analysis, whereas studies with an ambient temperature <30°C were excluded[22]. Therefore, we repeated our initial meta-analyses and added 12 recent per-cooling studies to our original approach (Table 2). On average per-cooling results in a 9.3% (ES=0.35) performance improvement (Figure 2), which did not differ from pre-cooling (5.7%, ES=0.44, p=0.32). Furthermore, per-cooling using cold water/ice slurry ingestion appeared to be the most effective strategy to enhance exercise performance (5.7%, ES=0.88), followed by an ice- or cooling vest (11.1%, ES=0.67), facial wind or water spray (18.5%, ES=0.54), cooling packs (4.4%, ES=0.33), and menthol cooling (8.7%, ES=0.23, Figure 2). These findings suggest that per-cooling is effective in improving exercise performance. However, one must realize that wearing a (heavy) ice vest (~1 kg)[50] or using facial wind or water spray cooling[57] may be feasible in laboratory conditions, but is generally not practical during competitive, field-based, settings.

It is important to note that we have found a discrepancy between the most effective cooling strategy based on the relative performance benefits and the effect size. An explanation for this finding may relate to the low number of per-cooling studies and subsequently low number of tested subjects. Hence, the effect-size is probably a better reflection of the true effects, as it allows a comparison across studies with different setups and sample sizes. Future per-cooling studies are needed to confirm the most effect per-cooling strategy.

The ambient temperature seems to impact the effects of per-cooling, as our analyses revealed a greater performance benefit in moderate (<30°C, 24.4±4.2°C) compared to hot (≥30°C, 32.3±1.9°C) ambient conditions (18.1%, ES=1.27 *versus* 5.9%, ES=0.28 respectively, p=0.015), whereas the effects of per-cooling in cold ambient conditions have not be investigated yet. This observation is unexpected, because the thermal load is larger in hot ambient conditions[58], which should facilitate the potential benefits of cooling. However, there is a large variation in performance benefits in the studies performed in moderate ambient conditions, ranging from a small non-significant negative effect (-0.6%, ES=0.08)[49] to a very large positive effect (51%, ES=1.17)[57] of per-cooling. This variation may possibly be explained by methodological differences in the exercise protocol and outcome measures, as a subjective outcome (rate of perceived exertion) was used as a surrogate for exercise intensity and may therefore influence the time to exhaustion[52]. Alternatively, the moderate training status of athletes that were included in these studies may contribute to the large within subject variability of performance benefits using facial water spray cooling[57].

Table 2. An overview of the studies using per-cooling in relation with exercise performance

Study	Exercise protocol	Method of Cooling	Change in exercise performance	Change in temperature	Ambient conditions	Conclusion
Ansley *et al.* 2008[46]	Cycling to exhaustion at 75% of VO_2 max	Facial water spray cooling (a spray every 30 s)	51% improvement in time to performance	No difference in Trec	29°C 50% rh	Facial water spray cooling improved exercise capacity
Barwood *et al.* 2015[42]	16.1-km cycling time trial	Menthol spray on cycling jersey after 10 km	No difference in time trial performance	No difference in Trec	33.5°C 33% rh	Menthol spray cooling did not improve time trial performance
de Carvalho *et al.* 2014[47]	40-km cycling time trial	Cold water ingestion (10°C, *ad libitum*)	No difference in time trial performance	No difference in Trec	35°C 60% rh	Cold water ingestion did not improve time trial performance
Cuttel *et al.* 2016[102]	Cycling to exhaustion at 60% of maximal power output	Ice vest during cycling	16.7% improvement in time to exhaustion	No difference in Trec	35°C 50% rh	Wearing an ice-vest is effective in improving exercise capacity, whereas a neck cooling collar is not effective
		Neck cooling collar during cycling	No difference in time to exhaustion	No difference in Trec		
Eijsvogels *et al.* 2014[36]	5-km running time trial	Cooling vest during exercise	No difference in time trial performance	No difference in Tgi	25°C 55% rh	Wearing a cooling vest did not improve time trial performance
Hsu *et al.* 2005[49]	30-km cycling time trial	Hand cooling (22°C) during cycling	6.6% improvement in exercise time	No difference in Ttymp	32°C 24% rh	Hand cooling improved 30-km cycling time trial performance
Luomala *et al.* 2012[37]	70 min cycling trial (60% VO_2 max) with intermittent-sprints (80% VO_2 max)	Ice vest applied after 30 min of exercise, until point of exhaustion	21.5% improvement of exercise time until exhaustion	No change in Tc	30°C 40% rh	Wearing an ice-vest during exercise enhances exercise performance

Study	Exercise protocol	Method of Cooling	Change in exercise performance	Change in temperature	Ambient conditions	Conclusion
Minetti *et al.* 2011[33]	90 min preloaded running (75 min 60% of VO_2 max and 15 min time trial)	Neck collar (-80°C, left in ambient conditions for 5 min before use)	11.3% improvement of covered distance during 15 min time trial	No difference in Trec	30°C 53% rh	Neck collar cooling is effective in improving exercise performance
Mündel *et al.* 2006[38]	Cycling to exhaustion at 65% of peak aerobic power	Cold water ingestion (3.6°C vs. 19.4°C)	11% improvement in time to exhaustion	Trec ~0.25↓ in second half of exercise protocol	33°C 28% rh	Cold fluid ingestion improved exercise capacity in the heat
Mündel and Jones. 2010[41]	Cycling to exhaustion at 65% of peak aerobic power	25 mL menthol ingestion every 10 min	8.6% improvement in time to exhaustion	No difference in Trec	34°C 27% rh	Menthol ingestion improved exercise capacity
Scheadler *et al.* 2013[48]	Running at 75% of VO_2 max until exhaustion	Hand cooling	11.6% impairment of exercise time until exhaustion	No difference in Tc	30°C 50% rh	Time to exhaustion was decreased by hand cooling
Schlader *et al.* 2011[39]	Cycling to exhaustion at RPE of 16	Facial wind cooling (20°C, 0.74 m/s)	17.8% improvement in time to exhaustion	No difference in Trec	20°C 48% rh	Facial wind cooling as well as menthol gel cooling improved time to exhaustion
		Facial menthol gel cooling (0.5 g/100 cm^2 of skin)	20.7% improvement in time to exhaustion	No difference in Trec		
Stevens *et al.* 2016a[40]	5-km running time trial	Facial water spray cooling (3 sprays every 0.2 km mark)	2.4% improvement in time trial performance	No difference in Trec	33°C 34% rh	Water spray cooling improved time trial performance

Study	Exercise protocol	Method of Cooling	Change in exercise performance	Change in temperature	Ambient conditions	Conclusion
Stevens *et al.* 2016b[43]	5-km running time trial	Menthol mouth rinse cooling (25 mL every 0.2 km mark)	2.8% improvement in time trial performance	No difference in Trec	33°C 46% rh	Menthol mouth rinse improved time trial performance
Teunissen *et al.* 2013[103]	15-km cycling time trial performance	Wind cooling (4 m/s) during kilometers 3-12	4.4% improvement in time trial performance	No difference in Trec	28°C 80% rh	Wind cooling improved time trial performance
Tyler *et al.* 2010[35]	**Study A:** 75 min running 60% of VO_2 max and a 15 min self-paced time trial	Neck collar (-80°C, left in ambient conditions for 5 min before use)	**Study A:** 5.9% improvement of covered distance during time trial	**Study A:** no difference in neck Tskin	30°C 50% rh	Cooling the neck can improve exercise performance in a hot environment.
	Study B: 15 min running time trial		**Study B:** no difference total covered distance	**Study B:** Neck Tskin is lower in cooling condition	30°C 50% rh	
Tyler and Sunderland 2011a[104]	90 min preloaded running trial (75 min 60% of VO_2 max and 15 min self-paced	Neck collar (-80°C, left in ambient conditions for 10 min before use)	7.0% improvement in time trial performance	Neck temperature is reduced by wearing a neck collar	30°C 53% rh	Neck cooling improved time trial performance
Tyler and Sunderland 2011b[34]	Running at 70% of VO_2 until exhaustion	Neck collar (-80°C, left in ambient conditions for 5 min before use)	13.5% improvement of exercise time until exhaustion	Neck Tskin is reduced Trec= 0.43↑	32°C 53% rh	Cooling the neck increased the time until exhaustion

Tc = core body temperature; Tskin = skin temperature; Trec = rectal temperature; Ttymp = tympanic temperature; Tgi = gastrointestinal temperature; VO2 max = maximal oxygen consumption; rh = relative humidity; RPE = rate of perceived exertion

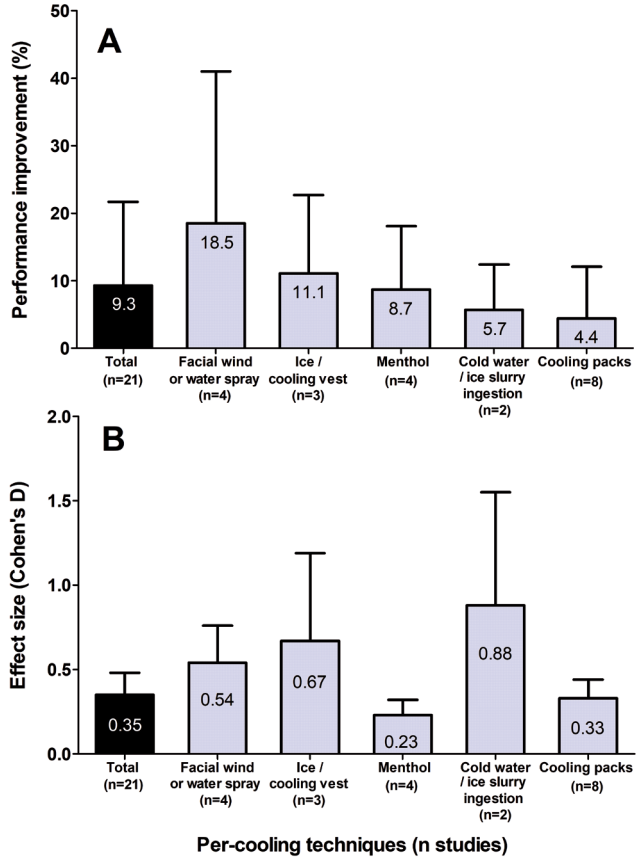


Figure 2. An overview of the average performance improvement (%) **(A)** and effect size **(B)** of per-cooling (black bar) and the beneficial effects of different per-cooling strategies (grey bars). Data are presented as mean ± standard deviation.

A few studies (n=5, 24% of per-cooling publications) found no difference in exercise performance between the per-cooling and control condition. An explanation may relate to the cooling power of the used interventions. One study used an evaporative cooling vest (~10°C)[49], while another study used cold water ingestion (10°C) to enhance performance[59]. The relatively high intervention temperature may be insufficient to elicit a performance benefit. Furthermore, the timing of cooling may explain the absence of an effect. In a menthol cooling study, the menthol spray was applied to the subjects cycling jersey after they covered 10 km (62%) of a 16.1-km cycling time trial[55]. Accordingly, the menthol spray could only impact performance for a relatively short period of time. Finally, the lack of an effect of per-cooling might be explained by the short exercise duration (15 min) of an experiment[48], in which increases in Tc were insufficient to attenuate exercise performance (peak Tc=38.4±0.3°C). Taken together,

'negative-studies' provide important information to researchers, coaches and athletes as they may aid in the selection of an appropriate cooling protocol.

Only 1 study demonstrated a substantial negative impact (-11.6%, ES=-0.17) of per-cooling on exercise performance[60]. In this randomized cross-over study, 12 subjects completed two time to exhaustion runs at 75% of VO_2 max with and without palm cooling in an ambient temperature of (30°C). Time to exhaustion was ~5.5 minutes longer in the control condition compared to the palm cooling condition. In contrast, another study reported that palm cooling improves 30 km cycling time trial performance in the heat (32°C) with 4 minutes (6.6%, ES=1.54)[61]. Therefore, the effects of palm cooling are still unclear and future studies are warranted.

Two studies explored the effects of per-cooling on resistance exercise performance. Intermittent palm cooling between four subsets of leg press resistance exercise resulted in a delayed decrement of average power output, resulting in a higher power output in the fourth subset of leg press exercise[62, 63]. A potential explanation for this finding may relate to temporarily overriding sensations of fatigue[62]. The peripheral thermal input may result in a lower awareness of effort while using palm cooling. Therefore, the motor output to contracting muscles is adjusted, by allowing less inhibition of the number of activated motor units, resulting in a higher power output and number of repetitions[63].

Concisely, the majority of per-cooling techniques are effective in improving exercise performance in the heat, in which cold water/ice slurry ingestion and wearing an ice-vest seemed to be most effective in laboratory conditions. It is recommended to use sufficient cooling power and continuous cooling exposure during the exercise trial to reach an optimal effect.

COMBINATION OF PRE- AND PER-COOLING

In addition to the studies that examined the effects of pre-cooling and per-cooling separately, some studies evaluated the combined effect of both types of cooling. We previously hypothesized that combining the advantages of pre-cooling and per-cooling should be more effective in improving exercise performance than a single cooling strategy[22]. Until now, 5 studies (with 9 individual comparisons) have examined the effects of a combination of pre- and per-cooling on endurance exercise performance. An overall improvement in exercise performance of 5.6% (range: -1.7% to +23%, ES=0.63) was found, which did not differ from pre- and percooling (p=0.23).

Only 1 study found a negative effect (-1.7%, ES=0.18) of using cold water ingestion (3°C) prior to and during a 20 km cycling time trial in the heat (31°C), whereas pre/per-cooling using an ice slurry (-1°C) or a combination of cold water or ice slurry with a menthol solution were

effective in improving performance (3.5%, 5.3% and 8.9% respectively, ES=0.50, 0.68 and 0.97)[64]. Furthermore, only 1 study was performed in moderate ambient conditions (28°C)[65]. Within this study, the ingestion of a menthol aromatized beverage of 3°C was not effective in improving exercise performance (3%, ES=0.32), whereas a menthol ice slurry (0.2°C) did significantly improve performance (6.2%, ES=0.67). This may suggest that vigorous cooling or a combination of cooling techniques may have a greater impact on performance, but there is no influence of ambient temperature on the potential benefits.

POST-COOLING

Post-cooling is defined as any opportunity to reduce the core, skin and/or muscle temperature directly after exercise, to enhance recovery from exercise and to reduce the exercise-induced muscle soreness. Different post-cooling interventions were described in literature[66], from which cold water immersion (5-15°C) is most frequently used. Furthermore, cold air exposure (-30°C) and local cooling using cooling packs were reported[67, 68]. Recently, (whole body) cryotherapy has been introduced as a popular post-cooling strategy. Cryotherapy involves exposures to extremely cold dry air (< -100°C) for short periods of time (2-4 minutes)[69, 70]. During cryotherapy individuals wear minimal clothing, gloves, a woolen headband covering the ears, a nose and mouth mask and dry shoes and socks to reduce the risk to develop cold injury[69].

In a recent meta-analysis, the effects of post-cooling were subdivided in subjective and objective outcomes for recovery[66]. It was found that post-cooling applied directly after exercise improves subjective recovery, since it lowers the symptoms of delayed onset muscle soreness after 24 and 96 hours of recovery[66]. Cold water immersion (5-15°C) appeared to be more effective compared to the other post-cooling strategies (cold air exposure, cooling packs and cryotherapy). Post-cooling also reduced subjective rate of perceived exertion after 24 hours of recovery, but not after 48 hours of recovery, whereas similar benefits were observed across different post-cooling techniques. In contrast, there was no evidence that post-cooling had an impact on objective recovery outcomes such as blood lactate, creatinine kinase and c-reactive protein concentration[66]. These findings were confirmed in a Cochrane review, which are recognized as the highest standard in evidence-based health care resources. Whole body cold water immersion did not impact maximal strength and maximal power output after 1 to 72 hours of recovery[71]. Moreover, no difference in biomarkers for muscle damage (creatine kinase) and inflammatory response (interleukine-6 and c-reactive protein) were found directly post-exercise and after 96 hours of follow-up[71].

Another Cochrane review focused solely on the effects of whole body cryotherapy on exercise recovery[69]. Four randomized controlled trials were included, with a total of 64 physically active predominantly male subjects. The cryotherapy intervention consisted of an exposure to an

ambient temperature ranging from -110°C to -195°C for three minutes. Results demonstrated lower levels of delayed onset muscle soreness after 1 hour, 24 hours and 48 hours of recovery in the cryotherapy condition compared to passive rest[69]. Objective improvements in recovery after cryotherapy were assessed using a maximal strength measurement. Interestingly, significantly greater maximal strength (range: 5.6% to 12.6%) was found at 24 to 120 hours after post-cooling compared to control[69]. Additionally, another study demonstrated that 5 days of cryotherapy (-110°C for 2 min) after a normal daily training program (3 hours) in highly trained athletes induced an increase in anti-inflammatory interleukin-10 and a decrease in the pro-inflammatory interleukin-8 and interleukin-2, suggesting that cryotherapy improves recovery after exercise by reducing the inflammatory response[72]. In contrast, 2 minutes of cryotherapy (-135°C) did not impact on plasma interleukin-6 levels after a competitive elite rugby match[73]. Although the effects of cryotherapy on the inflammatory response were not consistent, post-exercise cryotherapy seems to attenuate inflammatory response after exercise.

In summary, post-cooling lowers the subjective symptoms of delayed onset muscle soreness, in which cold water immersion is most effective (Figure 3). In contrast, cryotherapy did impact on objective recovery outcomes such as muscle strength and biomarkers for muscle damage in some studies, whereas cold water immersion did not impact on these objective outcomes. Additionally, cryotherapy may reduce the exercise-induced inflammatory response.

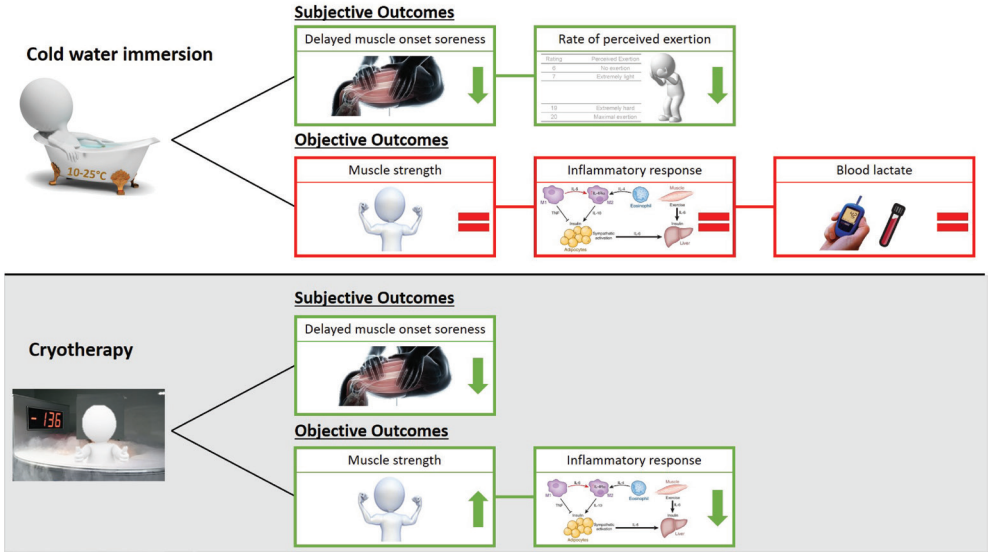


Figure 3. Overview of the effects of post-cooling on recovery from prolonged exercise, in which the effects were divided in subjective and objective outcomes. The 'arrows' represents a beneficial effects of post-cooling (↑ = higher, ↓= lower), whereas the '= sign' represents no impact of post-cooling.

THEORIES AND MECHANISMS FOR PRE- AND PER-COOLING BENEFITS

The basis of pre-cooling and per-cooling strategies is to reduce heat stress of the thermoregulatory system prior to and during exercise by increasing the heat storage capacity[17, 21]. Pre-cooling aims to lower Tc prior the onset of exercise, thereby increasing the margin for metabolic heat production and heat gain[17]. The pre-cooling-induced heat buffer enables athletes to perform more work before the critical limit for Tc is reached. Per-cooling aims to attenuate the exercise-induced rise in Tc, which delays the onset of hyperthermia-induced fatigue[22].

Critical core temperature theory

It has been shown that muscle power output, and thus heat production, is reduced by elevations in Tc[74]. The reduction in muscle power output is regulated by the central nervous system in order to protect the body to develop heat stroke[74, 75]. In fact, there may be a neural safeguard mechanism to terminate exercise once a critically high Tc (~40°C)[76] is obtained. This critical core temperature theory is supported by the observation that subjects quitted exercise at a similar Tc, but after dissimilar exercise durations, following repeated exercise bouts at different exercise intensities and starting temperatures[11]. Although the study subjects voluntary ceased exercise at a Tc of 40.1±0.1°C, the hyperthermia-induced fatigue should not be considered as an all-or-none action[77]. It appears to be more likely a dynamic process of progressive inhibition of the brain areas responsible for motor activation with increasing Tc that, together with sensory feedback from the exercising muscles and the cardiovascular system, provokes hyperthermia-induced fatigue during exercise in the heat[77, 78]. Therefore, a reduction in Tc prior (pre-cooling) or during (per-cooling) exercise may be effective in delaying the hyperthermia-induced fatigue. Pre-cooling predominantly results in a reduced Tc at the end of exercise compared to the control condition[22], whereas the majority of per-cooling research have found performance improvements without reductions in Tc[22]. Therefore, it is likely that other mechanisms are responsible for the beneficial effects of per-cooling.

Anticipatory theory

The rate of heat gain is continuously detected by our body, which could anticipatorily adjust the work rate to ensure that the exercise can be completed within the homeostatic limits of the body[18, 22]. More specifically, muscle activation during exercise in the heat may be reduced as a feed forward down-regulation of muscle drive as an anticipatory reaction to avoid the development of heat-related illnesses[79]. This anticipatory concept is supported by a previous study, in which subjects completed two 20 km self-paced time trials, one in the heat (35°C) and one in the cold (15°C)[79]. In the hot conditions, subjects reduced power output after 30% of time trial completion, whereas a similar phenomenon occurred at 50% of time trial completion in the cool condition. Furthermore, the rate of Tc increase was comparable between both conditions

(0.085±0.030°C/km *versus* 0.070±0.017°C/km, p>0.05)[79]. So, power output was adjusted well before reaching the critical limiting Tc. Therefore, the anticipatory response of the body may allow that exercise can be completed safely without the development of premature fatigue or heat stroke[79].

Core to skin temperature theory

Another mechanism that may contribute to the performance improvements following cooling relates to skin temperature and core to skin temperature gradient. This is confirmed by a recent study that examined time to exhaustion in four different groups runners that exercised under different ambient conditions (18°C, 26°C, 34°C and 42°C)[14]. A significant longer time to exhaustion was found in the 18°C and 26°C condition, with a greater core to skin temperature gradient but similar finishing Tc compared to the 34°C and 42°C condition[14]. Therefore, the core to skin temperature gradient has been identified as an important determinant for exercise performance in the heat, in which a larger gradient is advantageous for heat loss[80, 81]. The ability to sustain endurance exercise performance at a Tc above the critical Tc (>40°C) may be explained by the preservation of a cool skin temperature, which ensures a sufficient core to skin temperature gradient and the ability to stimulate heat loss[81]. These findings suggest that retaining a large core to skin temperature gradient may be even more important than keeping Tc below the critical Tc to preserve exercise performance. Therefore, any opportunity to reduce skin temperature prior to or during exercise may be beneficial to increase the core to skin temperature gradient and improve exercise performance.

Cardiovascular and Metabolic Mechanisms

Next to the direct effects of cooling on thermoregulation and exercise performance, cooling has also an indirect impact on performance via cardiovascular and metabolic mechanisms. Heat stress during endurance exercise is characterized by an increased metabolic[82] and cardiovascular strain[83]. Moderate heat strain is associated with a reduction in lactate threshold, which is a valid predictor for exercise performance in the heat[84]. The heat stress-induced downwards shift in lactate threshold as well as the increased blood lactate accumulation observed during heat stress may be an explanation for performance decrements during heat strain[38, 85]. Next to metabolic strain, heat stress induces an increase in skin blood flow to dissipate heat, which leads to a reduction in left ventricular stroke volume and limits muscular blood flow and oxygen delivery at the exercising limbs[83]. Cooling reduces the stress on the metabolic and cardiovascular system[19] as Tc reductions may inhibit blood lactate accumulation and increase the lactate threshold[38]. Furthermore, a lower Tc has been shown to reduce heart rate at a given workload[31, 32], and to reduce the cutaneous circulation that inhibits cardiac filling[38].

Psychophysiological Mechanisms

Several studies used menthol application to investigate the effects of changes in thermal perception without changes in Tc and skin temperature[54, 55]. Menthol is thought to stimulate a cool feeling [52, 56] via stimulation of cold receptors located in the skin[86] or oropharyngeal cavity[54]. The head and neck region appears to be the best area for menthol cooling[46, 48, 52], due to a greater density of cold-sensitive afferent thermal receptors[87]. Moreover, the mucous membranes of the oropharyngeal cavity are also sensitive for menthol[54]. As a result, oral application of menthol may enhance cold sensation in the mouth[88]. The menthol-induced cold perception may permit a higher self-selected exercise intensity and subsequent exercise performance improvement[86, 89]. Interestingly, a single menthol administration on the skin in resting conditions induces a greater increase in cutaneous vasoconstriction, rectal temperature and heat storage compared to oral menthol application and a control condition[90]. These results suggests that the modified perceptual signal is stronger than the physiological signal. As a result, the physiological system is overruled, leading to an increased metabolic rate and thus an increased heat production. Therefore, atlethes should be careful while using techniques that evoke a false thermal afferent signal as the discrepance between cold perception and actual Tc / skin temperature may increase the risk to develop heat-related illnesses.

PROPOSED MECHANISMS FOR POST-COOLING

In many sports, intensive training periods are alternated with strenuous competition phases, in which athletes have to maintain their best performance level for longer periods. Therefore, it is important to stimulate a fast recovery period between intense bouts of exercise. Exercise-induced physiological stress is related to hyperthermia, muscle damage, oxidative stress, inflammation and nervous system fatigue, which can result in a reduced performance potential[91]. This reduced capability to perform exercise might be explained by an increased muscle soreness and a decreased muscle function[92], a disturbed muscle reaction time or muscle stiffness that persists for several days[91, 93]. The application of cold directly after exercise (post-cooling) is often used to improve post-exercise recovery[66, 94]. Different proposed mechanisms for the recovery benefits of post-cooling were described, including a reduction in inflammatory response[95], a decrease in cardiovascular strain[96], and a decrease in muscle temperature and muscle damage[97]. However, the exact mechanisms through which post-cooling affects recovery from exercise are not well understood. Therefore, a number of suggested potential benefits of post-cooling on exercise recovery are described below.

Inflammatory response

Exercise induces metabolic stress in the active skeletal muscles, resulting in an increased generation of reactive oxygen species (ROS)[98]. ROS can denaturate proteins, nucleic acids and lipids, resulting in a destabilization of muscle cell structures such as the sarcolemma[99], and the excitation-contraction coupling system[100]. Damage to these structures modifies the muscle contraction kinetics, thereby reducing the force-generating capacity and exercise performance[101]. Furthermore, a destabilized sarcolemma makes the muscle fibers more permeable[101], which increases the potential to develop muscle fiber edema[102]. Edema increases the mechanical stress on muscle fibers, by impairing the oxygen delivery and waste removal, while it also causes muscle soreness[103]. Simultaneously with the muscle damage by ROS and muscle fiber edema, an exercise-induced inflammatory response is initiated that causes secondary muscle damage. This type of muscle damage is caused by inflammation in response to exercise and not the exercise *per se*, which results in muscle soreness and a lower muscle force generating capacity in the days after exercise[104]. Cold-induced vasoconstriction of the muscle vasculature and a decreased muscle tissue temperature due to post-cooling may cause reductions in cellular, lymphatic, and capillary permeability, which reduces the fluid diffusion into the interstitial space and decreases the risk of muscle fiber edema[91, 95]. Moreover, the decreased fluid diffusion, due to cooling, may assist in diminishing the acute inflammatory response to muscle damage. A lower inflammatory response is associated with less pain and lower decrease in muscle force generation[105]. Furthermore, the lower inflammatory response after post-cooling can be defined as an increase in an anti-inflammatory cytokine (IL-10), and a decrease in pro-inflammatory cytokines (IL-2, IL-8 and prostaglandin E_2)[106]. Therefore, post-cooling may have an anti-inflammatory response and may be effective in reducing secondary muscle damage, and may therefore enhance muscle recovery.

Cardiovascular & Thermoregulatory mechanisms

The implementation of post-cooling directly after a strenuous bout of exercise resulted in a faster reduction in heart rate and core, skin and muscle temperature[68, 107]. As a result of the faster decrease in heart rate, the cardiovascular strain during recovery is less. In addition, the rapid decrease in skin temperature, due to vigorous whole body cooling (cold water immersion or cryotherapy), causes a peripheral vasoconstriction of the skin. This leads to a diminished peripheral blood flow, resulting in a circulatory shift to the central blood circulation and a quick recovery of the central blood volume[69]. The augmented central blood volume and flow increases the ability of an athlete to remove waste products, such as lactate, and therefore may enhance recovery from exercise.

Additionally, post-cooling may immediately reduce the amount of muscle damage. Directly after exercise the muscle fibers are stressed, due to an increased energy demand to repair structural exercise-induced damage and replace energy stores[98]. The use of post-cooling

decreases the muscle tissue temperature, which causes a decrease in muscle metabolism and therefore a decrease in muscle energy demand[98]. Accordingly, the experienced metabolic stress by a muscle may be reduced due to a lower disparity between oxygen supply and oxygen demand. Furthermore, metabolic stress increases the mitochondrial energy production, which significantly contributes to the ROS production of a muscle cell[108]. Reducing the mitochondrial energy production by post-cooling may limit the ROS-medicated muscle damage after strenuous exercise. Therefore, it can be suggested that cooling may decrease the muscle stress directly after exercise, resulting in lower muscle soreness. Furthermore, some studies described an association between muscle tissue temperature and nerve conduction velocity[109, 110]. Post-cooling is known to reduce the sensory and motor nerve conduction velocity[111], which is associated with an increased pain tolerance and a decreased pain sensation[110]. Therefore, a post-exercise decrease in muscle temperature induced by cooling may have a temporary hypoalgesic effect, which attenuates the subjective perception of muscle soreness.

Taken together, post-cooling may enhance recovery from strenuous exercise by reducing the intramuscular temperature and muscle metabolism to reduce the metabolic stress and ROS generation associated with muscle damage, while local vasoconstriction may reduce the formation of edema, the inflammatory response and the associated secondary muscle damage. The subjective pain response to muscle soreness may be diminished by a cooling-induced decrease in nerve conduction velocity.

COOLING AND EXERCISE-INDUCED HYPERTHERMIA

Next to the effects of pre- and per-cooling on exercise performance, using cooling prior to or during exercise may also attenuate the increase in Tc and reduce the risk to develop heat-related illnesses. We previously described that the finishing Tc was lower in the cooling condition compared to the control condition for pre-cooling experiments (38.9 *versus* 39.1, p=0.03), but not for per-cooling experiments (38.9 *versus* 38.9, p=0.91)[22]. After adding recently published per-cooling studies to our initial analysis (Table 2), we still did not find a difference in finishing Tc between the per-cooling and control condition (38.7 *versus* 38.7, p=0.95). Based on these data, we may suggest that pre-cooling is effective in reducing thermal stress and lowering the final Tc, whereas it is not known whether pre- or per-cooling reduces the risk to develop heat-related illnesses. The greater metabolic work due to the cooling-induced performance benefit may also contribute to the comparable Tc between the cooling intervention and control conditions. Furthermore, one must realize that none of the included studies reported any heat-related disorders among their subjects. This suggests that our body is well able to use internal heat loss mechanisms to cope with an increase in Tc and to avoid critical high Tc that may lead to health problems.

PRACTICAL CONSIDERATIONS OF COOLING

The International Association of Athletics Federations World Championships of 2015 were held in Beijing, with expected ambient temperatures between 26-33°C[112]. In a cohort study preceding the World Championships, 957 athletes (49% of registered athletes) were included and asked to fill in a precompetition heat strategy questionnaire[112]. Based on the questionnaire, approximately 52% of the athletes have a prearranged pre-cooling strategy, from which ice slurry ingestion is most prevalent (24%)[112]. Additionally, approximately 47% of the athletes planned to use cold water immersion as a recovery strategy[112]. These findings highlight the popularity of cooling strategies in professional athletes, but also emphasize that more athletes could benefit from cooling interventions while competing in the heat.

The feasibility and applicability of implementing cooling interventions during training and competition is probably more important than its efficiency in improving exercise performance[21, 39]. The balance between efficiency and feasibility is reflected in the used pre- and post-cooling strategies applied during the Athletic World Championships[112]. Cold water/ice slurry ingestion and cold water immersion are not the most effective pre-cooling strategies, but can be easily applied in field based settings. In contrast, the use of per-cooling was not observed during this World Championchip. Internal cooling can be particularly suitable as a per-cooling strategy in competitive settings. However, a potential problem of these internal cooling methods is that the intake of large volumes of cold water/ice during exercise may cause gastrointestinal discomfort in some of the subjects[113]. Athletes should therefore experiment with the use of internal cooling during regular training sessions, to avoid any discomfort during competitive settings. An alternative easy applicable cooling intervention is the use of local cooling strategies. Cooling packs and evaporative cooling vests are very portable and can be implemented very easily prior to competition as a pre-cooling strategy. Moreover, local cooling as well as internal cooling have the practical benefit that it can be used simultaneously while fulfilling their normal preparations for competition. For post-exercise cooling strategies, cryotherapy may be an effective alternative to cold water immersion. However, it is important to use a maximal exposure duration of two to four minutes[73], since longer durations do not affect thermal and cardiovascular responses, but increase thermal discomfort of the subjects[114]. Furthermore, access to cryotherapy is limited, which make it less applicable for recreational athelethes.

CONCLUSION

Exercise-induced increases in Tc can negatively impact exercise performance and can lead to development of heat-related illnesses. The use of cooling techniques prior to, during or after exercise may attenuate the rise in Tc and may enhance exercise performance. Within

this review, we demonstrated that pre-cooling as well as per-cooling are effective in improving exercise performance in both moderate and hot ambient conditions. More specifically, using a mixed method pre-cooling strategy is most effective in improving exercise performance of athletes, whereas cold water/ice slurry ingestion is most favorable per-cooling strategy. Vigorous cooling techniques that cover a large part of the body, or techniques that can be applied frequently, appear to be the best for improvement of exercise performance. An overview of the benefits of cooling interventions is presented in Figure 4. The beneficial effects of pre-cooling and per-cooling may be explained by thermoregulatory as well as cardiovascular and metabolic mechanisms. Post-cooling is primarily focused on facilitating recovery after a strenuous bout of exercise, in which whole body cold water immersion is most effective in reducing the subjective rate of muscle soreness. Furthermore, cryotherapy may have a positive effect on objective outcomes of exercise recovery such as an increased maximal muscle strength and a decreased inflammatory response, whereas these effects were absent after cold water immersion. Taken together, any opportunity to reduce thermal strain prior to, during and/or directly after exercise is an effective strategy to improve time trial performance, exercise capacity and recovery from a stressful bout of exercise.

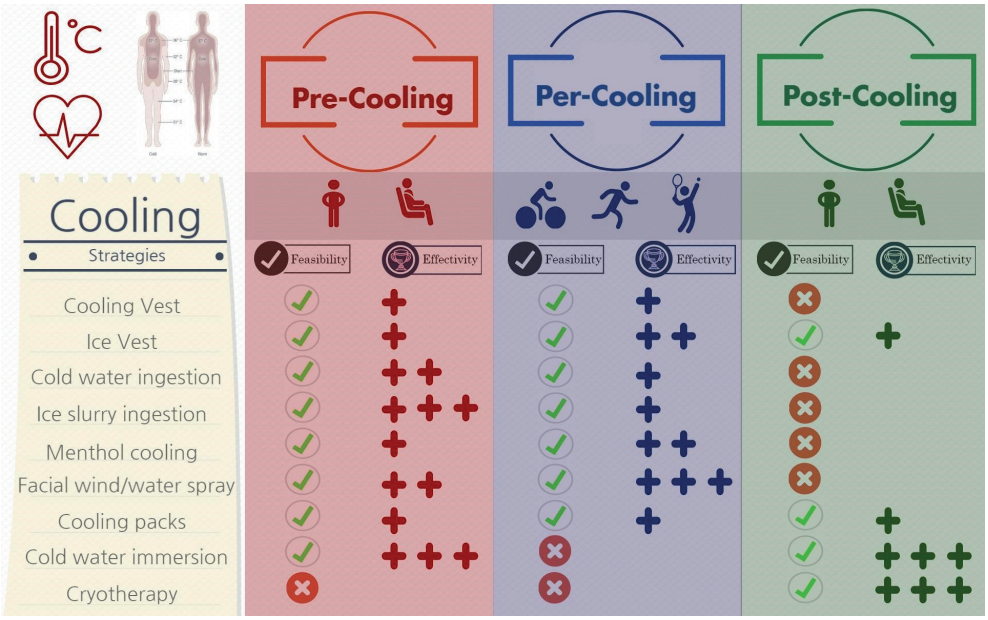


Figure 4. Infographic of the feasibility and effectivity of pre-, per- and post-cooling strategies. The effectivity of cooling techniques is classified as small (+), moderate (++) or large (+++).

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Chapter 7

Cooling during Exercise in Temperate Conditions: Impact on Performance and Thermoregulation

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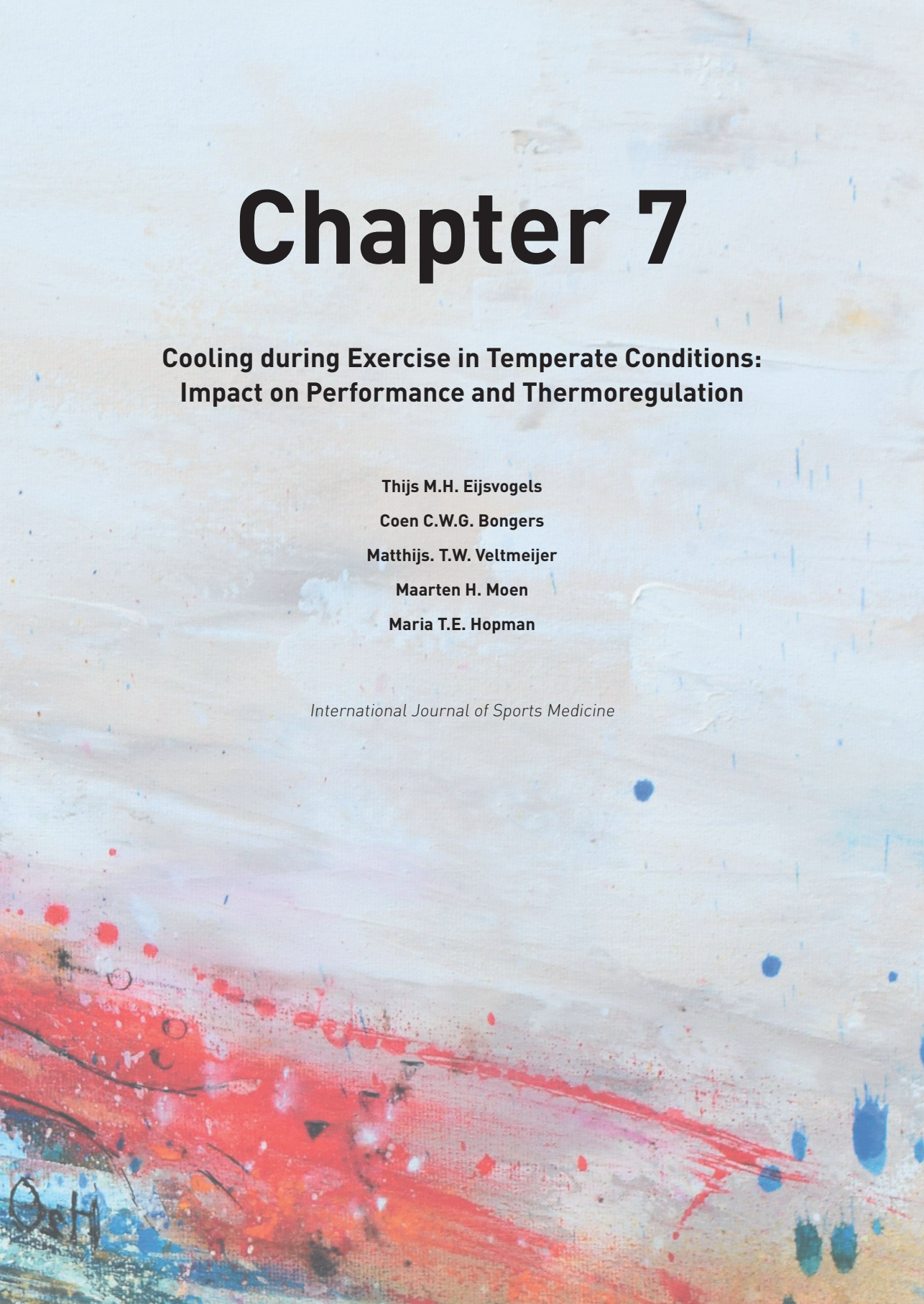


Cooling during exercise in moderate conditions: impact on performance and thermoregulation

10 Male trained athletes completed a 5 km running time trial in moderate conditions (25°C and 55% relative humidity)

Cooling

VS

Control

Cooling

Finish Time

20:54 (mm:ss)

Finish Temperature

39.0±0.3°C

Increase in Temperature

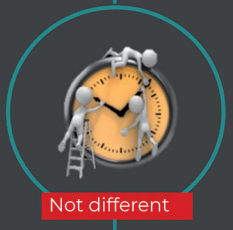
1.4±0.4°C

Finish Skin Temperature

34.4±1.5°C

Thermal Sensation

1.8±1.2



Control

Finish Time

20:46 (mm:ss)

Finish Temperature

39.1±0.5°C

Increase in Temperature

1.5±0.4°C

Finish Skin Temperature

34.6±0.9°C

Thermal Sensation

2.2±0.9

Wearing a cooling vest improves thermal comfort, but does not impact on performance or body temperature

ABSTRACT

Exercise-induced increase in core body temperature may lead to the development of hyperthermia (>40.0°C) and/or decreased performance levels. This study examined the effects of wearing a cooling vest during a 5-km time trial on thermoregulatory responses and performance. Ten male athletes (42±10 year) performed a 5-km time trial on a motorized treadmill in a climate chamber (25°C, 55% relative humidity) with and without cooling vest. Split times, heart rate, core-, skin- and cooling vest temperature were measured every 500 meters. Subjects also rated thermal comfort and rating of perceived exertion. The cooling vest significantly decreased heart rate ($p<0.05$), decreased skin temperature ($p<0.001$) and improved thermal comfort ($p<0.005$) during the time trial. Time to finish the 5-km time trial and pacing strategy did not differ between the control (1246±96 seconds) and cooling vest condition (1254±98 seconds, $p=0.85$). Also thermoregulatory responses, maximum core body temperature and rating of perceived exertion were not different across conditions ($p=0.85$, $p=0.49$, $p=0.11$, respectively). In conclusion, we demonstrated that wearing a cooling vest during exercise improves thermal comfort but does not enhance performance or decrease core body temperature in male athletes under temperate ambient conditions.

INTRODUCTION

The oxidation of substrates during running exercise results in muscle power (~20%) and heat production (~80%)[1, 2]. The increased metabolic heat production usually exceeds the maximal capacity of heat dissipation[3], which results in a rise in core body temperature (Tc). Accordingly, hyperthermia (Tc >40.0°C) may develop[3, 4], which could lead to decreased performance levels and/or the development of heat related illnesses[4-6]. Consequently, any attempt to delay the rise in core body temperature during exercise may enhance exercise performance levels in athletes, and prevent them from developing heat related symptoms[7-9].

In the last decade many cooling techniques were evaluated in athletes, with particular interest in pre-cooling strategies[10]. Pre-cooling increases the heat storage capacity of the body which enables an athlete to perform more work before reaching limiting Tc levels, thus delaying the onset of fatigue due to hyperthermia[9]. Pre-cooling with cold air, cold water immersion, cooling vests, ice slurry ingestion and combinations of these techniques effectively reduced Tc, and increased athletic performance levels in previous studies[5, 6, 11]. However, some of these cooling strategies (*i.e.* cold air / cold water immersion) may be impractical for use in competitive settings due to the need for specialized equipment, poor transportability to a field setting, athlete discomfort and costs[5, 11].

Cooling during exercise represents an alternative strategy to improve exercise performance. Previous studies indicated that local cooling of a small surface area (i.e. hand or neck) during exercise improved cycling and running performance by 6 to 13.5%[12, 13]. Cooling a larger body surface, such as using a cooling vest, may result in a further increase of exercise performance levels. Whilst previous cooling vests were uncomfortable and too heavy for athletes[14, 15], recent developments resulted in a new generation light-weight cooling vest (HyperKewl™) which is suitable for cooling during exercise. However, evidence of the benefits of wearing a cooling vest is currently restricted to pre-cooling studies only[10]. Therefore, the purpose of this study was to determine the effects of wearing a cooling vest during a 5-km treadmill time trial on performance and thermoregulatory responses in athletes. We hypothesized that a cooling vest is effective in limiting or delaying the increase in Tc, and subsequently may improve the time to finish the 5-km time trial. Interestingly, current regulations of the International Association of Athletics Federations (IAAF) allow the use of a cooling vest during race conditions[16].

MATERIALS & METHODS

Subjects

Ten male athletes volunteered to participate in this study (Table 1). Subjects were eligible if they were ≥18 years and had a 5 kilometer race personal record ≤20 minutes. Exclusion criteria were based on the use of the temperature pill: I) body weight <36.5 kg, II) implanted electro-medical device, III) gastro-intestinal disease, IV) a scheduled MRI scan. The study was approved by the Medical Ethical Committee of the Radboud University Nijmegen Medical Centre (study-id: 2011/546), and all subjects gave written informed consent prior to participation in the study. All procedures were in accordance with the ethical standards of IJSM[17].

Study design

In this randomized cross-over-design study, subjects were invited for four study visits. First, subjects were medically screened to determine whether they met the inclusion criteria. During the second visit, all subjects performed a habituation time trial: subjects performed the entire protocol and were able to get accustomed to running on a treadmill in the climate chamber (B-cat, Tiel, the Netherlands). Environmental conditions were controlled at an ambient temperature of 25°C, relative humidity of 55% and a wind velocity of 3 m/s, which is equal to an indoor WBGT index of 25°C. The experimental condition of the third and fourth visit were randomized to an intervention (cooling vest) or control time trial. All subjects had a minimum of 5 days of recovery between each visit. To remove any bias, subjects were informed that the study aimed to investigate whether running in a cooling vest either improved performances because of cooling, or decreased performances because of the added weight of the vest[9]. All visits were scheduled between 09.00 and 18.00. To minimize the effects of the circadian rhythm on the Tc and heart rate[14, 18], the time trial tests were performed at the same time of the day within subjects.

Table 1. Subject characteristics of the ten athletes included in the study

Variable	Subjects (n=10)
Age (years)	42 ± 10
Height (cm)	182 ± 5
Weight (kg)	73.6 ± 6.5
BMI (kg/m²)	22.2 ± 1.4
Personal record 5-km (min:sec)	18:10 ± 00:54

During all sessions, subjects were instructed to wear the same clothes, which consisted of a pair of shorts and a dry-fit running shirt. Subjects were allowed to eat and drink *ad libitum* before exercise, whilst they registered all fluid intake 24-h before the measurement. Furthermore,

subjects were instructed to take the same diet before each time trial to minimize the effect of nutrition. In preparation for all time trials, subjects were not allowed to perform strenuous exercise, ingest alcohol or caffeine 24 hours before testing as this may impact performance.

Time trial protocol

The 5-km time trial protocol is an effective method to demonstrate the effect of cooling interventions[14, 19]. The high exercise intensity ensures a rapid increase in Tc, which may impact performance and can potentially be counteracted by a cooling vest. Upon arrival in the climate chamber, body mass and baseline lactate level were measured. Rating of perceived exertion and thermal comfort were scored. Tc, skin temperature and heart rate recorders were applied, and data were obtained at baseline and every 500 meters during the time trial. The treadmill (Technogym excite med L1, Technogym, United Kingdom) was set at a 1% grade, to mimic conditions of outdoor road running[20]. Thereafter, subjects performed a standardized 12 minutes warm up: first speed was increased from 6 – 14 km/h (2 km/h steps per two minutes), followed by a cooling down at 10 km/h and 6 km/h (each 2 minutes). Subsequently subjects had 5 minutes for stretching and resting before the start of the time trial. In the intervention condition, the cooling vest was removed from the refrigerator and applied to the athlete 1 minute before the start of the time trial.

During the 5-km time trial, running speed was controlled by the subject. Information about running speed and split times was blinded for subjects, while completed distance was continuously displayed to assist with pacing. To obtain maximum performance, runners were verbally encouraged every 500 meter. Rates of perceived exertion and thermal comfort were scored every kilometer. Directly after completion of the time trial, body mass was determined again. Capillary lactate level was measured two minutes after finishing the 5-km time trial.

Cooling vest

The cooling vest (HyperKewl™, TechNiche, Vista, California, USA) was worn over the dry-fit running shirt and covered the major part of the subjects' trunk. The cooling surface area of the vest was 2258 cm^2. The day before each time trial, the cooling vest was activated according manufacturer instructions: 1) soak in water for two minutes, 2) squeeze excess water, 3) dry for two hours at room temperature. Then, the cooling vest was placed in a refrigerator (6.0°C ± 0.5°C, >8 hours) and ready to use. The weight of the activated cooling vest was 485 ± 85 grams.

Measurements

Split and finish times. Time to complete the 5-km time trial (finish time) was our primary outcome parameter. Also 500 meter split times were registered to detect potential differences in pacing strategy between the cooling vest and control condition.

Heart rate (HR). HR was measured at 15-second intervals using a Polar RS 400 system (Polar Electro Oy, Kempele, Finland). The highest HR value was presented as the HR_{max}.

Core body temperature (Tc). Tc was measured using a CorTemp™ system (HQ Inc., Florida, USA), which is safe and reliable[21]. Subjects ingested an individually calibrated telemetric temperature sensor at least five hours preceding the experiment, to avoid any interaction with fluid ingestion[22]. Tc was measured at 20-second intervals using an external recorder worn in a pouch around the waist.

Skin temperature (Tskin). Tskin was assessed using wireless temperature recorders (iButton DS1922L, Dallas Semiconductor Corp, USA) set to acquire temperature samples at 20-second intervals with a resolution of 0.0625°C[23, 24]. The temperature recorders were attached to the skin using Tegaderm film (Tegaderm, Neuss, Germany). Tskin was measured at eight distinct locations according to the ISO-9886 norm[25]. An index of Tskin mean was calculated as the weighted average of the 8 sites for each individual (Figure 1)[25].

Trunk temperature. We added two iButton sensors to assess the effect of the cooling vest more precisely. The average value of the 4 trunk iButtons was considered as the Tskin trunk (Figure 1)[25]. Differences between the Tc and Tskin trunk were expressed as the core to trunk temperature gradient, and calculated by subtracting these values.

Cooling vest temperature. Four iButtons were placed in the inside and outside fabric layers of the cooling vest (Figure 1). Cooling vest temperature was calculated using the average of these four locations. Cooling vest to Tskin trunk gradient was calculated by subtracting both values.
Blood lactate level. Capillary blood lactate levels were measured with an Accutrend plus GCT Cobas analyzer (Roche Diagnostics Limited, West Sussex, England). The blood lactate level was measured prior to warm up, and 2 minutes after finishing the 5-km time trial.

Subjective parameters. Thermal comfort was assessed on a 7-point category scale, in which -3 was corresponding with very cold and +3 was very hot[26]. The rating of perceived exertion was measured by the 10-point BORG category scale, in which 0 corresponded to rest and 10 to maximal exertion[27]. Both subjective parameters were scored every kilometer during the time trial.

Fluid balance. The relative change in body mass (in %) between the measurement at baseline and directly after completion of the 5-km time trial was calculated and dehydration was defined as a body mass loss of 2% or more[28, 29].

Data analysis

All values were presented as mean ± standard deviation, unless indicated otherwise. Statistical analyses were performed using SPSS (IBM SPSS version 20.0, Armonk, NY, USA.) and the level of significance was set at $p<0.05$. To assess differences in exercise characteristics between the control and cooling vest condition, a paired Student's T-Test was performed. To analyze differences over time during the 5-km time trial, and to determine whether physiological responses differed between the control and cooling vest condition we performed a two-way repeated measures ANOVA. Our statistical model included distance and condition (control or cooling vest) as within subject factors.

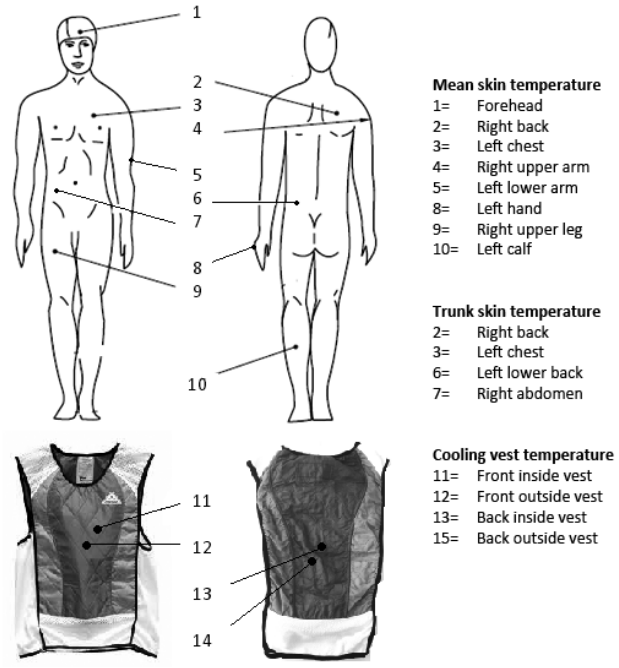


Figure 1. Overview of (anatomical) locations that were used to place the wireless iButtons sensors to measure the mean skin temperature, trunk skin temperature and cooling vest temperature.

RESULTS

Subject and exercise characteristics

All subjects successfully completed the 5-km time trials, while there were no differences in ambient conditions (temperature: p=0.19, humidity: p=0.32) between the cooling vest and control condition. The cooling vest significantly reduced in weight after the time trial (-50 gram,

p=0.003). Fluid loss was not different between conditions (p=0.19), with a body mass loss of -0.99±0.23% in the control condition and -0.96±0.24% in the cooling vest condition. Blood lactate levels significantly increased from baseline (2.2±0.5 mmol/L) to post-exercise (8.8±2.1 mmol/L, P<0.001), with no differences in the responses between both conditions (p=0.17).

Time trial performance and heart rate

The 5-km finish times were 1246 ± 96 seconds (20 minutes and 46 seconds) and 1254±98 seconds (20 minutes and 54 seconds) for the control and cooling vest condition respectively, and did not statistically differ (p=0.86) (Figure 2A). Furthermore, pacing strategy (expressed by split times) during the 5-km time trial was comparable across conditions (p=0.51). HR did not differ between conditions at baseline (p=0.96), and increased significantly during the 5-km time trial in both conditions (p<0.001). However, the average HR was significantly lower in the cooling vest compared to the control condition (Figure 2B, p=0.046). HR_{max} was 180±9 beats per minute in the control condition and 177±9 beats per minute in the cooling condition and did not statistically differ (p=0.11).

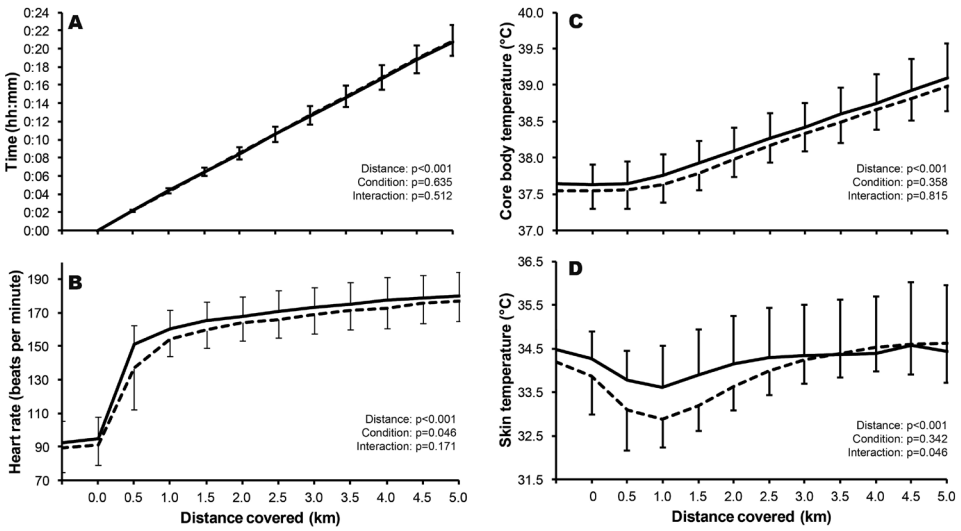


Figure 2. Performance levels, heart rate and thermoregulatory responses, in the control (solid line) and the cooling vest condition (dashed line) during the 5-km time trial. **(A)** Split times did not differ between conditions (p=0.64), while the pacing strategy was comparable (interaction; p=0.51). **(B)** Heart rate increased significantly during both trials (p<0.001). However, heart rate was significantly lower in the cooling vest compared to control condition (p = 0.046), despite a comparable course in heart rate over time (interaction; p = 0.17). **(C)** A significant increase in core body temperature was observed (p<0.001), with a comparable change over time across both conditions (p=0.82). **(D)** Skin temperature changed significantly throughout both conditions (p<0.001), with lower temperatures during the first half of the time trial in the cooling vest condition (p=0.046). The error bars represent the SD.

Core body temperature and skin temperature

Baseline Tc was 37.6±0.3°C during the control condition and 37.5±0.2°C during the cooling condition, and did not differ (p=0.18). Tc increased significantly during the 5-km time trial (p<0.001), with a comparable response across conditions (Figure 2C, p=0.82). Also the magnitude of the increase in Tc (p=0.85) and maximum Tc (p=0.49) did not differ between the control (1.5±0.4°C and 39.1±0.5°C, respectively) and cooling vest condition (1.4±0.4°C and 39.0±0.3°C, respectively). Tskin was comparable between the control and cooling vest condition (p=0.13) at baseline. Subsequently, Tskin changed significantly during the 5-km time trial (p<0.001), with significantly lower values in the cooling vest compared to the control condition (Figure 2D, p=0.046).

Trunk and cooling vest temperature

Tskin trunk was relatively stable in the control condition during the 5-km time trial, while a significant decrease was observed in the cooling vest condition (Figure 3A, p<0.001). Also, the Tskin trunk to Tc temperature gradient was higher in the cooling vest compared to the control condition (Figure 3B, p=0.004). Initial cooling vest temperature was 9.7±2.3°C and increased significantly during the 5-km time trial (Figure 3C, p<0.001). The cooling vest to Tskin trunk gradient showed an opposite curve with maximal difference of 24.6±2.2°C before the start, and 6.2±1.2°C upon completion of the time trial (Figure 3D).

Subjective parameters

The thermal comfort score was neutral at baseline, and significantly increased during the 5-km time trial (Figure 4A, p<0.001). While the change in thermal comfort score was comparable across groups (p=0.57), subjects reported an overall lower score in the cooling vest condition (p=0.003). Also, rating of perceived exertion scores increased significantly during the 5-km time trial (Figure 4B, p<0.001). However, absolute rating of perceived exertion scores (p=0.30) and the change over time (p=0.11) did not differ across conditions.

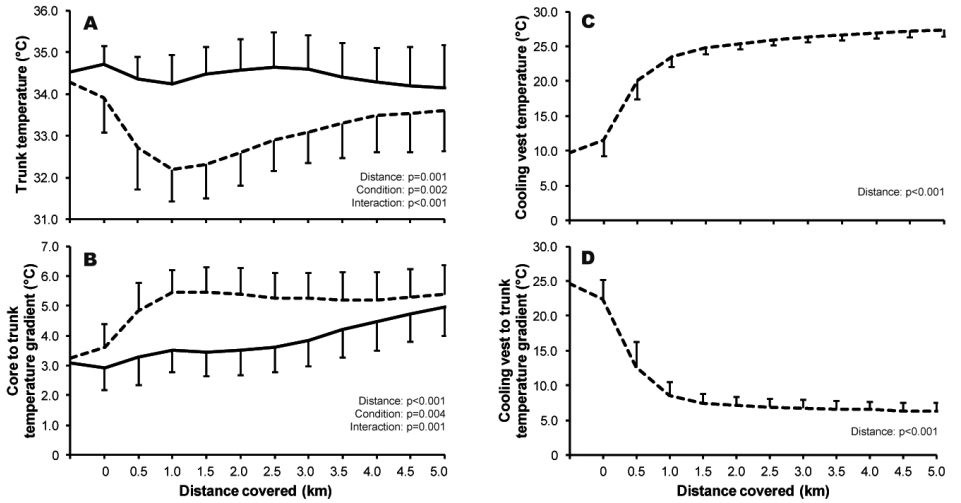


Figure 3. Trunk skin and cooling vest temperatures in the control (solid line) and the cooling vest condition (dashed line) during the 5-km time trial. **(A)** The change in trunk skin temperature was significantly different between conditions (p<0.001), with a marked decrease in temperature after the cooling vest was placed on the athlete (p=0.001). **(B)** Additionally, the core body to trunk skin temperature gradient demonstrated a different course over time between the conditions (p=0.001), with higher values in the cool vest condition (p=0.004). **(C)** Cooling vest temperature significantly increased during the time trial (p<0.001), while the cooling vest to trunk skin temperature gradient showed **(D)** an opposite response (p<0.001) with a value of 6.2°C at the end of the test. The error bars represent the SD.

DISCUSSION

This is the first study that assessed the effects of wearing a cooling vest during a 5-km time trial on performance levels and thermoregulatory responses. We found that wearing the cooling vest during exercise resulted in a significant decrease in skin- and trunk temperature, and an improved thermal comfort in masters athletes. Although the cooling vest resulted in a significantly lower HR, it did not improve the time to finish the 5-km time trial, nor did it affect Tc responses. These results suggest that wearing a cooling vest improves the comfort of athletes while running in ambient conditions of 25°C, but does not impact performance or Tc.

The use of a cooling vest to improve performance levels resulted in contradictory findings in precooling studies. While some studies found an improved time trial performance or exercise time until exhaustion[14, 30], others reported no difference between the cooling vest and control condition[9]. To our knowledge, we are the first to apply a light-weight cooling vest during running exercise in athletes. Despite a clear impact of the cooling vest on HR, Tskin and Tskin trunk, the split times and finish time did not differ across conditions. A potential explanation for these

findings may relate to the maximum Tc of 39.1°C that was observed. Previous studies suggested that exercise performance may be limited at a Tc of 40°C or higher[5, 6, 11]. Since our athletes did not reach the critical Tc threshold, they may therefore not have suffered from performance loss. Alternatively, the anticipatory hypothesis suggests that not peak Tc but the rate of increase in Tc is the limiting factor for performance decrement[5, 31]. Although subjects demonstrated a substantial Tc increase in this study, the cooling vest did not interact with Tc changes over time, potentially resulting in a comparable performance level in both conditions. Finally, fluid balance may also impact performance[32], but since subjects demonstrated a similar fluid loss in the control and cooling vest condition this explanation can be excluded. In summary, 1) a limited peak Tc or 2) a comparable rise in Tc but 3) not the fluid balance, may have contributed to the lack of differences in performance between the control and cooling vest condition.

An alternative explanation for the comparable performance levels can be found in the cooling capacity of the vest. Our evaporative vest had a baseline temperature of 9.7°C and a trunk to vest temperature gradient of 24.6°C before application of the vest to the athlete. However, after subjects covered only 1 km of the time trial, the vest temperature increased to 23.6°C and the gradient decreased to 8.6°C (Figure 3). Despite cooling vest temperature was still lower than Tskin, heat transfer was limited during this phase. These findings are reinforced by the HR of the athletes. Differences between conditions were large during this first km of the time trial (Figure 2B), but attenuated during the remainder of the test. A stronger cooling capacity of the vest may have prevented this. Indeed, studies that used an ice-vest found a large effect on performance and thermoregulatory responses[7, 33, 34]. However, the weight of the ice vest (1650 grams) is substantially higher compared to an evaporative vest[7]. Such heavy ice vests are therefore useful for precooling but inappropriate for cooling during exercise. Although the weight of our vest was low (489 grams), our data suggest a limited cooling capacity to significantly impact upon Tc. Future studies should therefore investigate the optimal relationship between cooling capacity and weight of the vest to guarantee maximal performance benefit for athletes during exercise.

Another factor that could contribute to our findings is the ambient condition under which the athletes had to perform the 5-km time trial. We chose a climate with an indoor WBGT index of 25°C. Although solar radiation cannot be simulated in a climatic chamber, we believe that these circumstances represent the ambient conditions that occur frequently during mass participation running events. These conditions can be classified as moderate / temperate[4], and therefore most studies that investigate the effects of cooling are performed in ambient temperatures of 30°C or higher[14, 30, 34]. Although the latter race-settings are relatively uncommon, all studies that were performed at these high ambient temperatures found a positive effect of the cooling vest on running performance. Indeed, a recent precooling studies demonstrated that cycling performance was enhanced in environmental temperatures of 30°C, but not at 25°C[7, 34, 35]. These results suggest that a cooling vest is predominantly effective

in high ambient temperatures, but may improve thermal comfort while exercising at lower ambient temperatures.

The strengths of the current study are the randomized cross over design and novel approach to apply a cooling vest during exercise. Moreover, we measured all important parameters that relate to performance and thermoregulation, which provided us detailed insight into the physiological responses during the time trial. However, some limitations should be taken into account. Firstly, the circadian rhythm of the Tc could influence thermoregulatory responses during exercise[18]. Nevertheless, we have successfully anticipated to that by scheduling the 5-km time trials at the same time of the day resulting in a comparable baseline Tc (p=0.40) between the control (37.6±0.3°C) and cooling vest condition (37.5±0.2°C). Secondly, an inherent problem with (pre-)cooling studies is the inability to blind the subjects for the intervention, which could bias their performance. To remove any potential bias of our study design, we instructed all athletes that our primary goal was to test if the cooling capacity of the vest overrules the additional weight of wearing the vest during the 5-km time trial. Accordingly, the cooling vest could either have a positive (cooling) or negative (more weight) effect on the 5-km time trial performance. Finally, the cooling power (watts) of the vest is currently unknown, which limits the direct comparison with other cooling techniques.

The results of this study indicate that wearing a cooling vest during exercise is not effective in improving running performance in male competitive runners under temperate ambient conditions. Furthermore, wearing a cooling vest does not affect Tc during exercise. In contrast, the cooling vest did result in a lower HR, lower Tskin and improved thermal comfort in our mater athletes. Also, the additional weight of the cooling vest did not negatively impact on finish or split times. These findings suggest that wearing a cooling vest may be comfortable during practice, although the cooling vest does not enhance performance. Future research should determine the optimal cooling capacity *versus* weight of a vest that is worn during exercise. Combining our findings with data from previous studies suggest that a lightweight fabric with long-lasting and (ultra) low temperatures might be the optimal cooling strategy for competitive athletes.

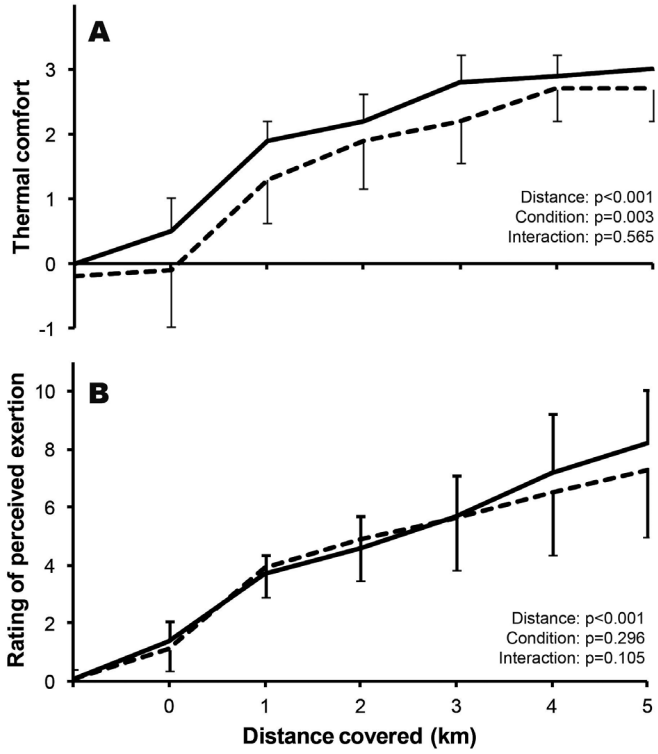


Figure 4. Thermal comfort and rating of perceived exertion scores in the control (solid line) and the cooling vest condition (dashed line) during the 5-km time trial. **(A)** Thermal comfort scores increased significantly in both conditions ($p<0.001$), however the overall score was lower in the cooling vest compared to the control condition ($p=0.003$). **(B)** The rating of perceived exertion increased significantly during the 5-km time trial ($p<0.001$), with a comparable response across conditions. The error bars represent the SD.

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Chapter 8

The Effects of Cooling During Exercise on Thermoregulatory Responses of Men with Paraplegia

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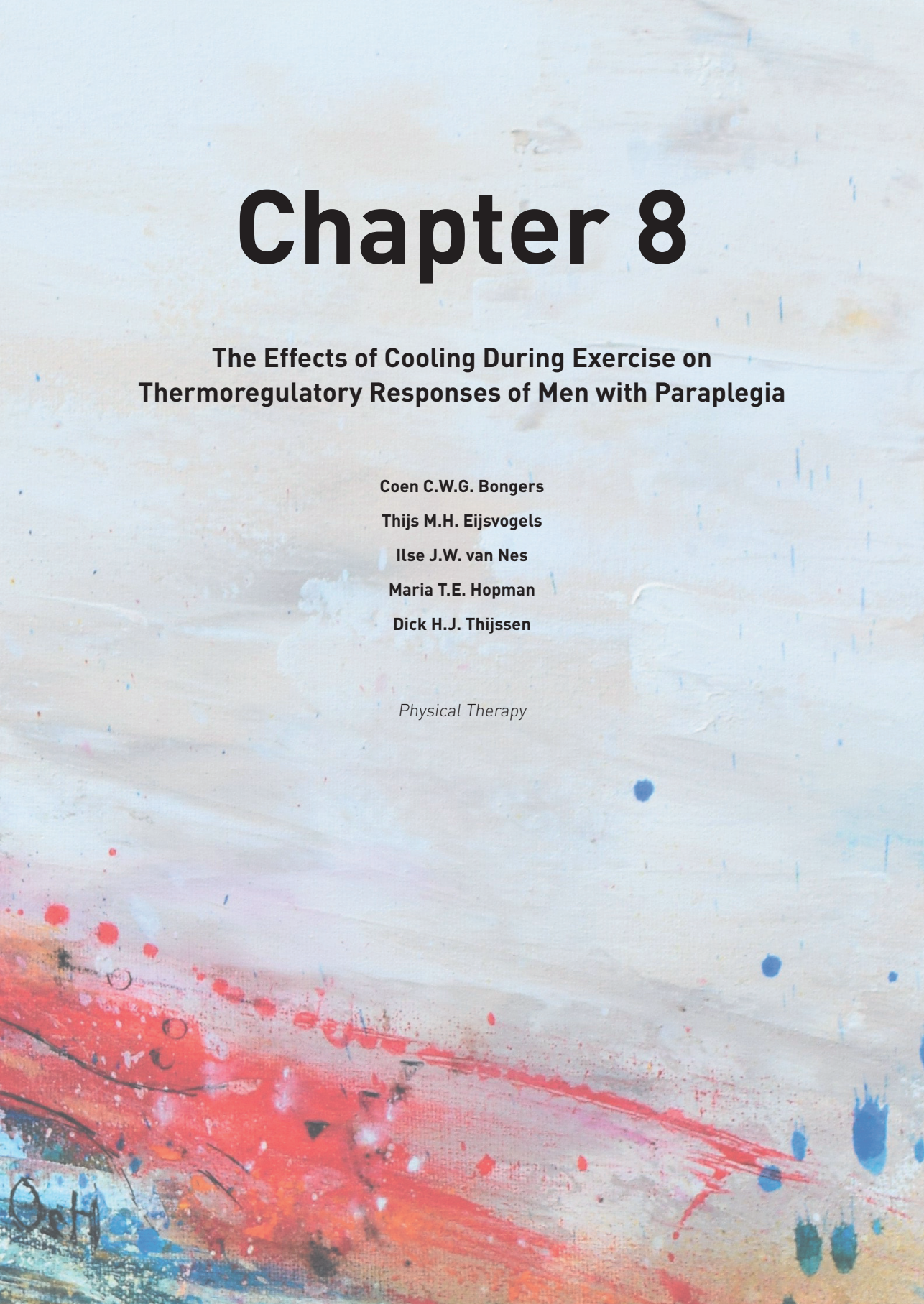
Physical Therapy



Cooling during exercise on thermoregulatory responses of men with paraplegia

10 Men with a thoracic spinal cord injury completed two exercise protocols in moderate conditions (25°C, 41% relative humidity)

45 min at 50% of maximal workload

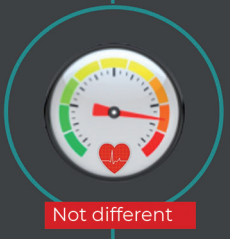
Cooling

VS

Control

Cooling

Control



Not different

Exercise Intensity

83±7%

Exercise Intensity

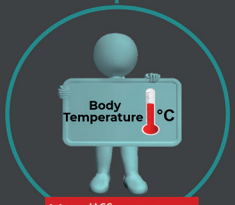
79±6%

Finish Temperature

37.9±0.1°C

Increase in Temperature

0.8±0.1°C



No differences, except for Tskin

Finish Temperature

37.8±0.4°C

Increase in Temperature

0.9±0.1°C

Finish Skin Temperature

32.5±0.3°C

Finish Skin Temperature

33.1±0.4°C

Thermal Sensation

0.9±1.2

Thermal Sensation

1.3±1.1

A cooling vest lowers skin temperature and thermal sensation, but does not impact on core body temperature

ABSTRACT

Individuals with spinal cord injury (SCI) have an altered afferent input to the thermoregulatory center, resulting in a reduced efferent response (vasomotor control and sweating capacity) below the level of the lesion. Consequently, core body temperature (Tc) rises more rapidly during exercise in individuals with SCI compared to able-bodied subjects. Cooling strategies may reduce the thermophysiological strain in SCI. The aim of this study was to examine the effects of wearing a cooling vest on Tc response of subjects with a thoracic SCI during sub-maximal exercise. Ten men (44 years; SD= 11 years) with a thoracic lesion (T4/T5 or below) participated in this randomized crossover study. Subjects performed two 45-minute exercise bouts at 50% maximal workload (ambient temperature 25°C) with subjects randomized wearing a cooling vest or no vest (separate days). We continuously measured core body temperature and skin temperature, whilst thermal sensation was assessed every 3-minutes. Exercise resulted in an increased Tc, skin temperature, and thermal sensation ($p<0.05$), whereas cooling did not impact Tc ($p>0.05$). The cooling vest effectively decreased skin temperature ($p<0.001$), increased core-to-trunk skin temperature gradient ($p=0.003$) and tended to lower thermal sensation ($p=0.07$) compared to the control condition. Despite effectively lowering skin temperature and increasing core-to-trunk skin temperature gradient, we found no impact of the cooling vest on the exercise-induced increase in Tc in men with low SCI.

INTRODUCTION

Individuals with spinal cord injured (SCI) have an impaired sensory and motor function, which is often associated with damage to the autonomic nervous system causing a reduced afferent input to the thermoregulatory center[1, 2] and an impairment of the efferent system leading to attenuated sweating response and vasomotor control below the level of the lesion[3-7]. These characteristics result in an impaired thermoregulation in subjects with SCI, especially under highly demanding conditions[3, 8, 9]. Thermoregulatory strain typically occurs during prolonged exercise in challenging ambient conditions, which leads to an increased core body temperature (Tc)[10].

A recent meta-analysis showed that cooling strategies prior to (pre-cooling) or during exercise (per-cooling) effectively attenuates the increase in Tc during a bout of prolonged exercise of 60 minutes[11, 12] in able-bodied athletes[13]. Accordingly, cooling strategies prior to or during exercise may also be effective in individuals with SCI. A previous meta-analysis demonstrated conflicting results of per-cooling (using ice vest-, foot-, head- and neck cooling) on the thermoregulation of individuals with SCI[14]; while some studies demonstrated a lower core body temperature using per-cooling techniques during intermittent sprint or continuous exercise[15-18], others found no effects[19, 20].

The conflicting results of per-cooling interventions during intermittent sprint and continuous exercise may relate to the weight of the vest and/or comfort level[15, 19]. Taking these limitations into consideration, a new light-weight evaporative cooling vest was developed (HyperKewl™), which is designed for use during exercise. In able-bodied athletes, this cooling vest effectively decreased the skin temperature and improved the thermal sensation during exercise[21]. To date, no previous study explored the effectiveness of this cooling vest on thermoregulatory responses during exercise in individuals with SCI. Therefore, we examined the effects of wearing a cooling vest during 45 min of moderate-intensity exercise on the core temperature, skin temperature, core-to-trunk skin temperature gradient as well as on thermal sensation in individuals with SCI. We hypothesized that the cooling vest is effective in attenuating the exercise-induced increase in Tc through markedly increasing the core-to-trunk skin temperature gradient.

METHODS

Study population

10 Male subjects with paraplegia volunteered to participate in this study (Table 1). The level of the thoracic lesions ranged between T4 and T12, with ASIA impairment scale (AIS) scores of A and B (8 subjects AIS=A; 2 subjects AIS=B). Furthermore, all subjects were >1 year post-injury and were able to perform arm crank exercise for at least 45 minutes (VO2max= 24.0 ml/min/kg;

SD= 6.5 ml/min/kg, Table 1). Exclusion criteria were based on the use of the temperature pill and include: I) body weight <36.5 kg, II) implanted electro-medical device, III) gastro-intestinal disease, and/or IV) a scheduled MRI scan. All subjects gave written informed consent prior to participation in the study, and the study was approved by the Medical Ethical Committee of the Radboud university medical center. Furthermore, all procedures were in accordance with the guidelines of the Declaration of Helsinki.

Table 1. Subject characteristics and the results of the maximal exercise test

Subject	Age (years)	Body mass (kg)	Height (cm)	Level of injury	Years-post injury	PPO (W)	VO_2max (L/kg/min)	HRmax (bpm)	Lactate (mmol/L)	50% PPO (W)
1	43	66.7	183	T6-T7 T6*,	20	150	32.8	180	8.8	75
2	36	60.8	173	T11-12	9	80	17.7	182	2.8	40
3	49	68.5	186	T12-L1	26	130	29.2	189	13.0	65
4	52	98.9	187	T10-T11	10	130	21.8	132	9.1	65
5	26	72.5	173	T6*	6	120	26.4	189	11.3	60
6#	32	72.5	182	T4/T5	12					80
7	55	80.0	178	T8	18	70	16.2	148	7.9	35
8	51	92.1	179	T9/T10	29	80	17.3	115	5.9	40
9	32	72.0	174	T10-L1	16	160	32.6	188	13.7	80
10	59	86.0	172	T8-T9	26	90	21.8	147	7.1	45
Mean	43.5	77.0	179		17.2	112	24.0	163	8.8	59
SD	11.3	12.0	6		8.0	33	6.5	28	3.5	17

PPO= peak power output; VO_2 max= maximum oxygen consumption; HR max= maximum heart rate. * Incomplete lesion representing AIS score B; # Subject did not perform a maximal exercise test; peak PO was estimated.

Study design

In this randomized cross-over study, subjects were invited for 3 study visits. The first visit consisted of obtaining the signed informed consent form, performing a medical screening to check whether subjects were eligible for participation, and performing a maximal arm crank exercise test to assess peak oxygen uptake and peak power output (peak PO). During the randomized second and third visit, subjects performed a 45-minute sub-maximal arm crank exercise test at 50% of peak PO with or without cooling vest. Prior to exercise, the subjects were allowed to drink and eat *ad libitum*. However, subjects were instructed to eat the same diet prior to both testing days to minimize the effects of nutrition. Furthermore, subjects registered their fluid intake 24 hours prior to the test and they were instructed to wear the same clothes during both tests. All subjects had at least 5 days of recovery between each visit.

To minimize the effects of the circadian rhythm on Tc and heart rate (HR)[22], we scheduled both sub-maximal exercise tests consistently at the same time of the day within each subject. Finally, subjects were not allowed to perform severe exercise or consume alcohol or caffeine 24 hours before all study visits, as this may impact the exercise test.

Day 1: *Maximal exercise test*

Physical fitness (peak oxygen uptake, VO_2max) and peak PO were determined using a maximal exercise test on an arm ergometer (Angio Cycle Ergometer, Lode, Groningen, the Netherlands) in an ambient temperature of 19°C. During the maximal exercise test, subjects started cycling at 10 Watt with an arm crank cycle frequency of 60-80 rpm. Workload increased every minute with 10 Watt until voluntary exhaustion. Continuous measurement of oxygen uptake (VO_2) and carbon oxide output (CO_2) was performed using an automatic gas analyzer (Quark CPET v9.1b, Cosmed, Rome, Italy). Peak oxygen uptake was calculated as the average oxygen uptake during the last 30 seconds of the test. Peak power output (peak PO) was defined as the workload at the highest intensity that the subject could maintain for at least 30 seconds. HR was measured continuously using a 12-lead ECG.

Day 2-3: *Sub-maximal exercise test*

During the second and third visit, subjects performed a 45-minute sub-maximal arm crank exercise test at 50% of their individual peak PO. Both tests were performed in a temperature controlled chamber with an ambient temperature of 25.4°C (SD= 0.4°C) and a relative humidity of 41.0% (SD= 8.4%). Ambient temperature during both sub-maximal exercise tests was higher compared to the maximal exercise test to induce a higher thermal stress. Ambient temperature was similar between both testing days to ensure valid assessment of the potential cooling effect of the vest. Upon arrival, body mass was measured in the sitting position and recorders to assess Tc, skin temperature (Tskin) and HR were applied. Prior to exercise, a baseline measurement of 10 minutes in the sitting position was taken to obtain baseline values of Tc, Tskin and HR. Thereafter, subjects performed a standardized warm-up with workload being increased from 30 to 50 Watt (10 Watt per 2 min), followed by 2 minutes at 40 Watt. Subsequently, a fan was turned on and subjects had 2 minutes for stretching and resting before the start of the sub-maximal exercise test. The fan was placed 140 cm in front of the subjects and the speed of the fan was 1.44 m/s. Air circulation was created by turning on the fan to mimic real-life conditions and facilitate the cooling capacity of the evaporative cooling vest. After the warm-up, the cooling vest was applied (for the intervention visit). Then, the exercise test was started and subjects were instructed to exercise at a frequency of 60-80 repetitions per minute. In the second sub-maximal exercise test the same protocol was used to ensure that thermal load was comparable between both tests. After completing the sub-maximal exercise, the workload was, depending on the peak PO, decreased to 20-40 Watt and subjects performed a cool-down for 5 minutes. Finally, subjects rested for 10 minutes, during which

the cooling vest was removed and the fan was turned off. Throughout the test, Tc, Tskin and HR data were obtained every 5 minutes, whilst the rate of perceived exertion and the thermal sensation were scored every 3 (during exercise) or 2 minutes (during warm-up, cool-down and recovery). After completing the protocol, post-exercise body mass was determined to assess exercise-induced fluid loss.

Intervention: Cooling vest

In this study a light weight, evaporative cooling vest was used as cooling intervention. The cooling vest (HyperKewl™, TechNiche, Vista, California, USA) covered the major part of the subjects' trunk. The cooling vest contains a water management system, which consists of 3 layers that stimulate water absorption and storage. Subsequently, the evaporation process of the water contributes to cooling of the skin. The cooling surface area of the evaporative cooling vest was approximately 2294 cm^2 (SD= 134 cm^2). The day before each time trial, the cooling vest was applied according manufacturer instructions: 1) soak in water for 2 minutes, 2) squeeze excess water, 3) left the vest for 2 hours at room temperature to dry the outside of the vest, and 4) Place in a refrigerator (5.8 °C ± 0.2°C) for at least 10 hours. The weight of the activated cooling vest was 388 gram (SD= 67 gram).

Measurements

Core body temperature (Tc). Tc was measured using an ingestible temperature pill (CoreTemp™ system, HQ Inc., Florida, USA), which is a safe and reliable technique[23]. Subjects were instructed to ingest the individually calibrated telemetric temperature pill at least 5 hours preceding the measurement to avoid any interaction with fluid ingestion[24]. During the protocol, Tc was measured every 20 seconds using an external recorder and data was presented every 5 minutes. For safety reasons, the sub-maximal exercise test was terminated when the core body temperature exceeded 40°C.

Skin temperature (Tskin). Tskin was examined using wireless temperature sensors (iButton DS1922L, Dallas Semiconductor Corp, USA). The configuration of the sensors was set to collect data at 20-seconds intervals with a resolution of 0.0625°C[25]. Furthermore, all skin temperature data were presented every 5 minutes during the exercise test. The sensors were placed on the skin using Tegaderm Film (Tegaderm, Neuss, Germany), and Tskin was measured at 8 different locations according to the ISO-9886 standard[26] (Figure 1). An index of mean Tskin was calculated as the weighted average of the 8 sensors for each subject, which was based on the relative surface of the body area that each measuring point represents[26].

Trunk skin temperature. To assess the effects of the cooling vest more precisely, we added two additional thermal sensors to the chest. The average value of the 4 sensors (2 from the standard 8-point placement and the 2 additional sensors) placed on the trunk was considered

as Tskin trunk (Figure 1). Differences between Tc and Tskin trunk were expressed as the core-to-trunk skin temperature gradient, which was calculated by subtracting these values.

Cooling vest temperature (Tvest). Four additional sensors were placed on the inside and outside fabric layers of the cooling vest (Figure 1) to obtain the cooling vest temperature. Tvest was calculated as average of these 4 measurement locations. Vest-to-trunk temperature gradient was calculated by subtracting these values.

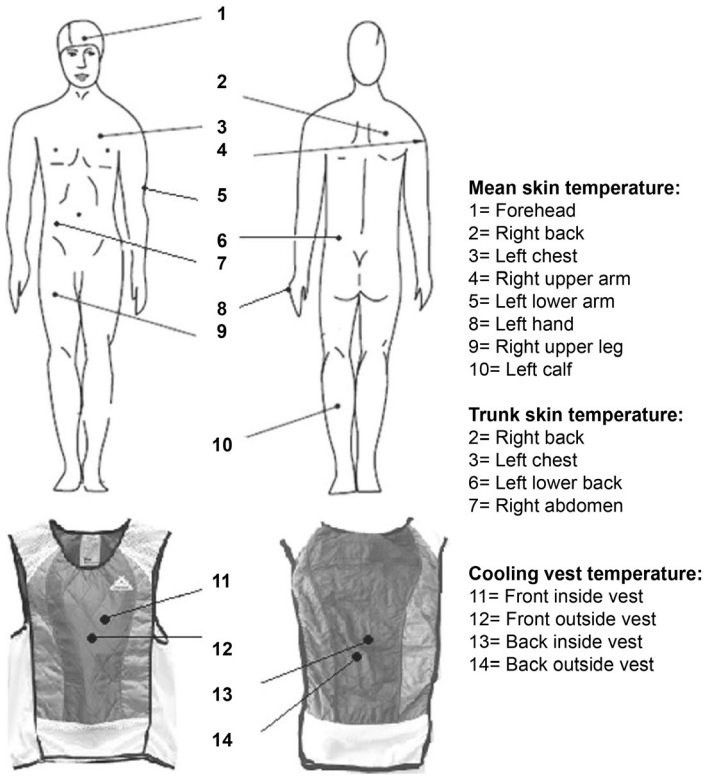


Figure 1. Overview of (anatomical) locations that were used to place the wireless iButton sensors to measure the mean skin temperature, trunk skin temperature and cooling vest temperature.

Heart rate (HR). HR was continuously monitored during exercise on 15 seconds intervals using a Polar RS400 system (Polar Electro Oy, Kempele, Finland). Data was presented every 5 minutes and the highest HR was presented as the HRmax. The exercise-induced increase in HR (ΔHR) was calculated by subtracting the HR during exercise by the HR at baseline.

Fluid balance. The relative change in body mass (in %) between the pre- and post-exercise body mass was calculated. Dehydration was defined as a body mass loss of 2% or more[27]. *Rate of perceived exertion and thermal comfort.* The rate of perceived exertion was scored using a 10-points Borg scale, in which 0 corresponded to rest and 10 to maximal exertion[28]. Thermal sensation was scored using a 7-points category scale, in which -3 represents very cold and +3 was very hot[29]. Both subjective parameters were scored and presented every 3 minutes during the sub-maximal exercise and every 2 minutes during warm-up, cool-down and recovery.

Statistical analysis

All values are presented as mean (standard deviation, SD), unless indicated otherwise. Statistical analyses were performed using Statistical Package for Social Sciences 20.0 (IBM SPSS version 20.0, Armonk, New York, USA) and the level of significance was set at $p<0.05$. To assess differences in exercise characteristics (Table 2) between the cooling and control condition, a paired Students' *t*-test was performed and data were presented as mean (SD) and as mean difference including a 95% confident interval (MD (95%CI)). A two-way repeated measures ANOVA was used to examine differences in physiological responses (Tc, Tskin, Tskin Trunk, HR, RPE and thermal sensation) over time between the cooling and control condition, in which 'time' and 'intervention' were used as within subject factors.

RESULTS

Subject and exercise characteristics

Subject characteristics and the results of the maximal exercise test are shown in Table 1. Average exercise intensity, ambient temperature and relative humidity were 81.3% (SD= 10.1%), 25.4°C (SD= 0.4°C) and 41.0% (SD= 8.4%), respectively, and did not differ between both testing days (Table 2). None of the subjects exceeded the Tc safety limit of 40°C during exercise and none of the subjects met the criteria for being dehydrated after completing the test (>2% loss in body weight). During exercise, HR increased in both conditions ($p<0.001$). Interestingly, throughout the exercise protocol, HR was significantly higher in the cooling condition ($p=0.012$), whilst the workload was exactly the same in both protocols. Peak heart rate was significantly higher in the cooling condition (148 bpm; SD= 26 bpm) compared to the control condition (142 bpm; SD= 27 bpm, $p=0.013$), with a mean difference (MD) of 6 bpm (95% CI= 2 bpm, 12 bpm)). In contrast, ΔHR throughout exercise did not differ between conditions ($p=0.46$, MD= 3 bpm (95% CI= -5 bpm, 10 bpm).

Thermoregulation

Core body temperature. At baseline, Tc was comparable between the cooling (37.1°C; SD= 0.3°C) and the control condition (37.0°C; SD= 0.3°C, $p=0.45$, MD=0.1°C (95% CI= -0.2, 0.4°C). Tc

increased significantly during both sub-maximal exercise tests (p<0.001) and the increase was comparable between conditions (Figure 2A). We found no differences between the cooling vest and control intervention for maximum Tc (37.8°C; SD= 0.1°C *versus* 37.9°C; SD= 0.1°C, p=0.47, MD= 0.1°C (95% CI= -0.2°C, 0.3°C)) nor for the increase in Tc (0.9°C; SD= 0.1°C *versus* 0.8°C; SD= 0.1°C, p=0.90, MD= -0.02°C (95% CI= -0.3°C, 0.3°C)).

Tskin. Baseline Tskin was comparable between cooling (32.5°C; SD= 0.9°C) and control condition (32.8°C; SD= 1.0°C, p=0.33, MD= -0.3°C (95% CI= -1.0°C, 0.4°C)). We demonstrated a significant increase in Tskin over time during exercise, with significantly lower values in the cooling condition compared to the control condition (Figure 2B). In addition, maximum Tskin was lower during the cooling condition compared to the control condition (33.1°C; SD= 0.4 *versus* 32.5°C; SD= 0.3°C, p=0.04, MD=-0.6°C (95% CI= -1.2°C, -0.03°C)).

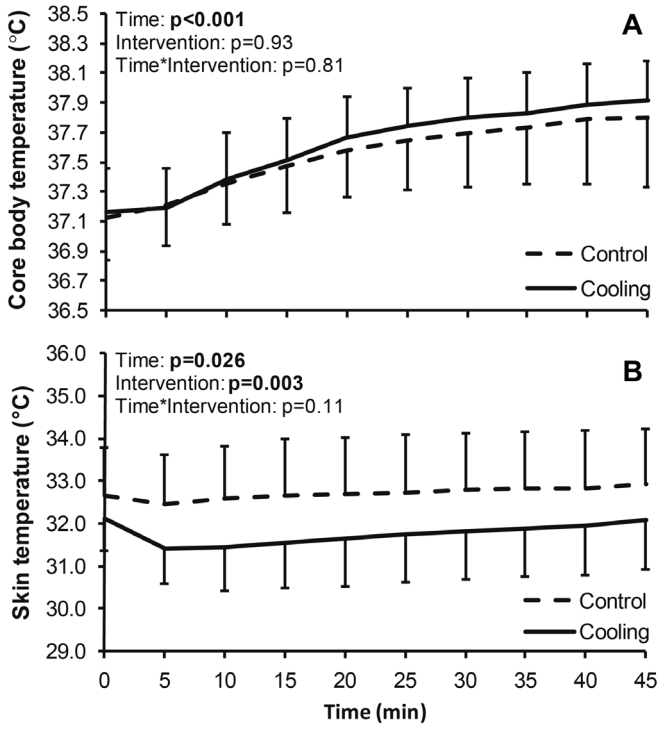


Figure 2. Core body temperature and skin temperature responses during the sub-maximal exercise test for the cooling (solid line) and the control (dashed line) condition. **(A)** A significant increase in core body temperature was observed (p<0.001), with a comparable change over time across both conditions (p=0.81). **(B)** The skin temperature was significantly lower in the cooling condition compared to the control condition (p=0.003). The data were presented as mean (SD) (n=10).

Ttrunk. Trunk skin temperature significantly increased over time (p=0.008), with a lower increase in the cooling vest condition compared to the control condition (p=0.010, Figure 3A). Furthermore, the core-to-trunk temperature gradient was higher in the cooling condition compared to control (p=0.003, Figure 3B).

Tvest. Directly after applying the cooling vest the Tvest was 12.2°C (SD= 6.1°C), whilst Tvest increased during exercise (p<0.001, Figure 3C). Vest-to-trunk skin temperature gradient decreased during exercise (p<0.001) from 17.1°C (SD= 2.8°C) to 6.5°C (SD= 1.1°C) (Figure 3C).

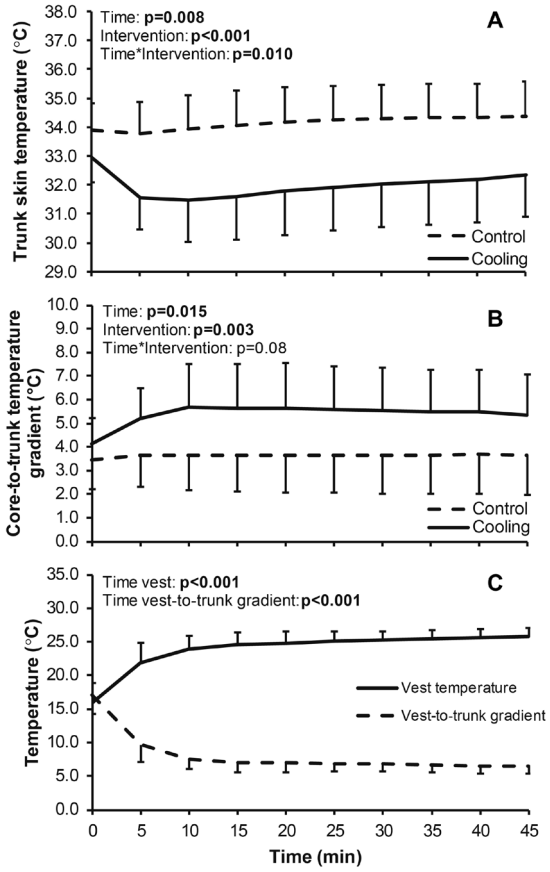


Figure 3. Trunk skin and cooling vest temperature in the cooling (solid line) and control condition (dashed line). **(A)** The trunk skin temperature increased during exercise (p=0.008) and was lower in the cooling condition (p=0.01). **(B)** The core-to-trunk temperature gradient was higher in the cooling condition compared to control (p=0.003). **(C)** Additionally, the cooling vest temperature increased during the sub-maximal exercise (p<0.001), whilst the vest-to-trunk temperature gradient decreased during exercise (p<0.001). The error bars represented the SD (n=10).

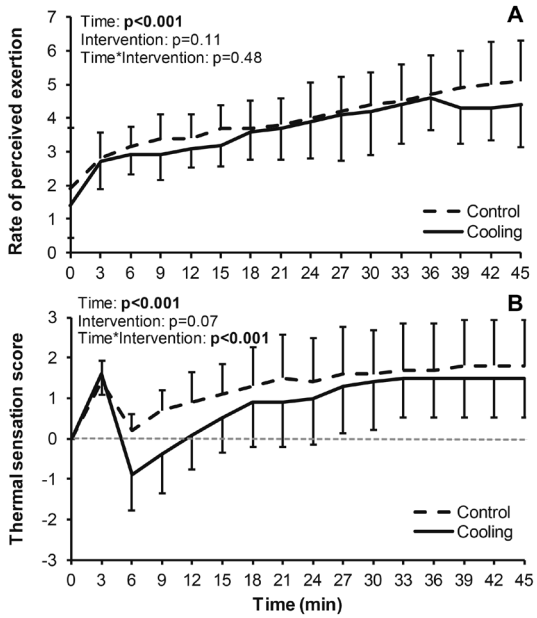


Figure 4. Rate of perceived exertion **(A)** and thermal sensation **(B)** response during sub-maximal exercise in the cooling (solid line) and control condition (dashed line). Data were presented as mean (SD) (n=10).

Subjective parameters

Sub-maximal exercise caused an increase in rate of perceived exertion and perceived thermal sensation during exercise for both groups (Figure 4A-B). However, the increase in thermal sensation score was different between conditions (p<0.001), with subjects reporting a trend for a lower thermal sensation in the cooling condition (p=0.07). Additionally, the rate of perceived exertion did not differ between conditions (p=0.11).

Table 2. Exercise characteristics sub-maximal exercise test

Outcome parameter	Control	Cooling	p-value
Exercise intensity (%)	79.3 (6.2)	83.4 (6.5)	0.40
Ambient temperature (°C)	25.5 (0.4)	25.4 (0.5)	0.75
Relative humidity (%)	43.3 (8.5)	38.6 (8.0)	0.22
Baseline Tc (°C)	37.0 (0.3)	37.1 (0.3)	0.45
Peak Tc (°C)	37.8 (0.4)	37.9 (0.3)	0.47
Baseline HR (bpm)	76 (13)	80 (14)	0.11
Peak HR (bpm)	142 (27)	148 (27)	**0.013**
Δ HR (bpm)	66 (25)	68 (26)	0.41
Body mass loss (kg)	-0.33 (0.08)	-0.29 (0.09)	0.11
Body mass loss (%)	-0.4 (0.1)	-0.4 (0.1)	0.15
Dehydration; >2% body mass loss (n(%))	0 (0%)	0 (0%)	1.00

Tc= Core body temperature; HR= heart rate. Data were presented as mean (SD).

DISCUSSION

In this study we aimed to examine the impact of wearing a cooling vest during prolonged exercise on the thermoregulatory responses of individuals with a low thoracic SCI. We demonstrated that wearing a cooling vest during exercise did not affect the exercise-induced increase in Tc neither the maximum Tc in individuals with a low thoracic SCI. Nonetheless, we found that the cooling vest during exercise resulted in a lower Tskin, an increased core-to-trunk temperature gradient and tended to improve thermal sensation in individuals with SCI. Despite these effects of the cooling vest on skin temperature and subjective parameters of thermal comfort, our results suggest that percooling using a light weight evaporative cooling vest is not effective in attenuating the increase in Tc in individuals with paraplegia when performing 45 minutes of moderate-intensity exercise under moderate climate conditions.

The cooling vest did impact the exercise-induced increase of core body temperature. A possible explanation for the absence of an effect of cooling on core body temperature could be the relatively moderate ambient conditions in which the exercise tests were performed. Previous studies showed beneficial effects of wearing a cooling vest in ambient temperatures of 30°C and higher, but not at temperatures ≤25°C[15, 16, 20]. Although the use of an ambient temperature of 25°C is on the lower end of the spectrum, maximal Tc in our study is comparable to those observed in previous studies that were performed under higher ambient temperatures[15-17, 19]. Furthermore, in a study by Armstrong and colleagues[19], a comparable increase in aural temperature during exercise was shown after ice vest cooling under challenging conditions (32.9°C). This may suggest that the relatively moderate ambient temperature as adopted in our study unlikely fully explains the lack of an effect of the cooling vest on the exercise-induced increase in Tc in individuals with a low thoracic SCI.

Studies that demonstrated differences in thermoregulatory response between cooling and control conditions have typically included individuals with SCI with lesions between C1 and T5[15-17]. In these group of subjects, a large area of sensate skin, including both afferent information on thermal state and efferent responses to dissipate heat, is being affected[3]. This provides room for cooling strategies to impact core body temperature[30, 31]. Indeed, the rise in Tc during exercise is proportional to the level of the lesion[6, 32-34], which makes individuals with SCI with a cervical or high thoracic lesion more susceptible to develop a large increment in Tc during exercise. This is in line with a study by Griggs *et al.*[35], in which a higher increase in Tc was found in tetraplegic (C4-C7) compared to paraplegic (T4-S1) individuals. Nine of our subjects reported a lesion level <T6, suggesting the presence of a relatively normal thermoregulatory and sweating response. The relatively low lesion level in our study may be an explanation for the absence of an effect of cooling. However, in studies with abled-bodied subjects, the effects of wearing a cooling vest during exercise were also absent[21, 36].

An alternative explanation for the comparable Tc during exercise in both conditions could be the cooling capacity of the evaporative cooling vest. Directly after applying the cooling vest the Tvest was 12.2°C (SD= 6.1°C) and the vest-to-trunk skin temperature gradient was 21.6°C (SD= 6.1°C). However, after 10 minutes of exercise the Tvest increased to 23.9°C (SD= 2.0°C) and the vest-to-trunk skin temperature gradient was decreased to 7.6°C (SD= 1.6°C) (Figure 4C). Although the cooling vest temperature was still lower than Tskin, heat exchange and cooling capacity of the vest were markedly lower compared to the start of exercise bout. A higher cooling capacity may contribute to a larger impact on the vest-to-trunk temperature gradient and, consequently, be able to affect Tc during exercise.

The lack of an effect of the cooling vest on core temperature may also be explained by the location of cooling. Previous work demonstrated that arteriovenous anastomoses, which are mainly located in the distal part of the extremities, have an important role in the heat exchange with the environment[37]. Under warm conditions, arteriovenous anastomoses stimulate heat loss by supplying blood to the upper parts of the skin. As a result heat conduction to the environment will be enhanced and cooled blood will be returned to the core[17, 38]. Active manipulation of the arteriovenous anastomoses using cooling techniques may be highly effective in heat removal in rest and during exercise[39]. Therefore, the lack of an effect of the cooling vest may relate to the focus of cooling central regions, whilst not changing the (possibly more important) distal areas rich of arteriovenous anastomoses.

In addition to Tc responses we also explored whether the cooling vest intervention had an impact on Tskin. As a result of the damaged autonomic nervous system, the ability to vasoconstrict and vasodilate the peripheral vasculature is diminished in individuals with SCI[7, 9, 31]. Hence, there will be no vasoconstriction of the cutaneous blood vessels below the level of the lesion, while wearing a cooling vest, allowing for a greater thermoregulatory effect of the cooling vest. Indeed, we found a lower Tskin in the cooling condition as well as a higher core-to-trunk skin temperature gradient. These findings demonstrate that the cooling intervention effectively impacts the thermoregulation in individuals with a low thoracic SCI. However, the reduction in Tskin was insufficient to attenuate the exercise-induced increase in Tc.

An unexpected finding of the present study is the higher HR in the cooling condition compared to the control condition. Workload, ambient conditions and time of the day were comparable between conditions and, therefore, unlikely to contribute to this difference in HR. Interestingly, when correcting for individual differences in resting HR by presenting the change in HR from baseline (=ΔHR), we found a comparable increase in HR during exercise between both conditions. Therefore, differences in HR during exercise may be explained by potential differences in baseline HR. Furthermore, we found no difference between both exercise tests for RPE, whilst the cooling vest condition was associated with a lower perceived thermal

sensation. Therefore, it may be suggested that individuals with SCI felt more comfortable while wearing a cooling vest during sub-maximal exercise in moderate ambient conditions.

The strengths of this study are the randomized crossover design and novel approach to using a light weight cooling vest during exercise in subjects with a low thoracic lesion. Still, some limitations should be taken into account, because we did not measure sweat rate using sweat sensors. However, measuring changes in body mass may represent a good alternative for sweat loss, especially since subjects were not allowed to drink or go to the toilet. Interestingly, we found no difference in weight loss between both conditions (-0.4±0.1% *versus* -0.4±0.1%), suggesting that sweat loss was not different between trials. Furthermore, we were not able to blind the subjects for the type of intervention (with or without cooling vest), which may possible result in a placebo effect. However, we tried to minimize the placebo effect by keeping the subjects naive about the potential positive or negative effect of cooling.

In conclusion, in this study we demonstrated that wearing an evaporative cooling vest during a 45-minute sub-maximal arm crank exercise is not effective in limiting or delaying the increase in Tc in individuals with SCI with a low thoracic lesion. Nonetheless, the cooling vest improved the perception of thermal sensation and decreased the Tskin. These findings may suggest that wearing a cooling vest may be comfortable for SCI individuals during exercise in moderate ambient conditions, despite the fact that it does not impact core body temperature. Whether the cooling vest has any potential impact on exercise performance should be subject for further research.

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Chapter 9

Thermoregulation and Fluid Balance During a 30 km March in 60 versus 80-Year Old Subjects

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Age

Thermoregulation and fluid balance during a 30-km march in 60- versus 80-year-old subjects

40 sexagenarians (60±1 years) and 36 octogenarians (81±2 years) participated in a 30-km walking march

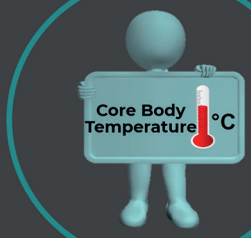
Exercise intensity

30 km

Sexagenarians

Finish Temperature
38.2°C ↓

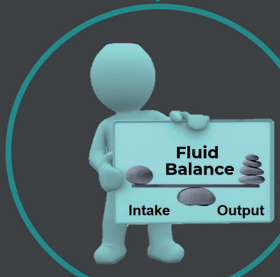
Increase in Temperature
0.7°C ↓



Fluid Intake
325 mL/h ↑

Urine Output
52 mL/h ↑

Sweat Rate
364 mL/h ↑



Overall body mass loss = -0.7%

Octogenarians

Finish Temperature
↑ 38.4°C

Increase in Temperature
↑ 1.2°C

Fluid Intake
↓ 251 mL/h

Urine Output
↓ 28 mL/h

Sweat Rate
↓ 294 mL/h

Overall body mass loss = -1.2%

Thermoregulatory responses deteriorate with advancing age, whereas fluid balance control did not

ABSTRACT

The presence of impaired thermoregulatory and fluid balance responses to exercise in older individuals is well established. To improve our understanding on thermoregulation and fluid balance during exercise in older individuals, we compared thermoregulatory and fluid balance responses between sexagenarians and octogenarians during prolonged exercise. 40 Sexagenarians (60±1 year) and 36 octogenarians (81±2 year) volunteered to participate in a 30 km march at a self-selected pace. Core body temperature (Tc) and heart rate were recorded every 5 km. Subjects reported fluid intake, whilst urine output was measured and sweat rate was calculated. Octogenarians demonstrated a lower baseline Tc and a larger exercise-induced increase in Tc compared to sexagenarians (1.2±0.5°C *versus* 0.7±0.4°C, $p<0.01$), whilst maximum Tc tended to be higher in octogenarians (38.4±0.4°C *versus* 38.2±0.3°C, $p=0.09$). Exercise intensity (70±11% *versus* 70±9%) and exercise duration (7h45min±0h57min *versus* 7h24min±0h58min) were not different between octogenarians and sexagenarians. Octogenarians demonstrated lower fluid intake (251±97 mL/h *versus* 325±125 mL/h, $p=0.01$) and urine output (28±22 mL/h *versus* 52±40 mL/h, $p<0.01$) compared to sexagenarians. Furthermore, the sweat rate tended to be lower (294±150 mL/h *versus* 364±148 mL/h, $p=0.07$) in the octogenarian group. Sodium levels and plasma volume changes were not different between sexagenarians and octogenarians (all $p>0.05$). These results suggest that thermoregulatory responses deteriorate with advancing age, while fluid balance is regulated appropriately during a 30 km walking march under moderate ambient conditions.

INTRODUCTION

The increased metabolic heat production during exercise leads to a rise in core body temperature[1, 2] and may ultimately lead to the development of heat-related illnesses[3]. The primary heat dissipating mechanism during exercise is evaporation through sweating, accounting for 80% of total heat loss[4, 5]. However, the production of sweat in combination with insufficient fluid replacement may lead to the development of dehydration[6] and could impair exercise performance and increase the risk to develop hyperthermia[7].

Advanced age is associated with a negative impact on thermoregulatory and fluid balance responses during exercise[8, 9]. Previous studies, which compared young *versus* old subjects, showed that elderly ≥60 years have a lower baseline core body temperature, a reduced skin vasodilatory capacity, a less effective sweat response, and a decreased sensitivity of the thermal receptors[8-10]. Furthermore, advanced age is associated with a decreased total body water[11, 12], decreased thirst sensation[13, 14], decline in kidney functioning[11, 15] and a reduced plasma vasopressin regulation at rest and during dehydration[16, 17]. These characteristics make older humans a vulnerable population for the development of hyperthermia and dehydration[8, 9, 17-19]. Most of this knowledge is derived from cross-sectional observations between cohorts of young (usually <30 years) and older humans (usually >60 years). Accordingly, it is unknown whether thermoregulation and fluid balance control deteriorates further with aging or plateaus at some point.

The purpose of our study was, therefore, to compare thermoregulatory responses and fluid balance during prolonged, moderate-intensity exercise between sexagenarians and octogenarians. We hypothesized that exercise-induced thermoregulatory responses and fluid balance control are further deteriorated in octogenarians compared to sexagenarians.

METHODS

Subjects

A total of 79 subjects volunteered to participate in this study (Table 1). Subjects were recruited via a newsletter, which was distributed by the organization of the Nijmegen Four Days Marches (an annual walking event in The Netherlands). Based on the age classification of previous publications, subjects were divided into the group of sexagenarians (60±1 year, 24 men and 16 women) or octogenarians (81±2 year, 25 men and 14 women). The following subjects were excluded from participation: I) body mass<36.5 kg, II) implanted electro-medical device, III) a history of gastro-intestinal disease or abdominal surgery, and IV) a scheduled MRI-scan within 1 week after participating in the present study (all based on the use of the temperature pill). The study was approved by the Medical Ethical Committee of the Radboud university medical

center, and all subjects gave written informed consent prior to participation. This study was conducted in accordance with the Declaration of Helsinki.

Experimental design

Baseline measurements, including subject characteristics, body composition, urine specific gravity and blood levels of sodium, hemoglobin and hematocrit, were performed in our temperature controlled laboratory one or two days prior to the exercise bout. The blood pressure was measured in supine position after lying on a bed for 5 minutes, whilst a blood sample was taken in sitting position. Immediately before the start of exercise, subjects underwent assessment of body mass, heart rate (HR), and core body temperature (Tc). Thereafter, subjects participated in the first day of the Nijmegen Four Days marches and walked 30 km at a self-selected pace, all starting at 7.00 AM. The exercise bout consisted of a 30 km self-paces march on a flat course over tarmac roads. During exercise, Tc and HR were recorded after every 5 km, while urine excretion was collected using collecting bags and all subjects registered their fluid intake using a diary. Directly after finishing, subjects reported to our laboratory to determine post-exercise body mass, HR, Tc, fluid balance (urine and blood analysis).

Measurements

Subject characteristics. Body mass (Seca 888 scale, Hamburg, Germany) and body height were measured and body mass index (BMI) was calculated. A four-point skin fold thickness measurement (biceps, triceps, sub-scapular, supra-iliac) was performed in order to calculate the lean body mass[20]. Waist circumference was measured midway between the lower rib margin and iliac crest. Hip circumference was measured at the level of widest circumference over greater trochanters. Waist-to-hip ratio was calculated as waist circumference divided by hip circumference. Thereafter, resting heart rate and blood pressure were measured twice using an automated sphygmomanometer (M5-1 intellisense, Omron Healthcare, Hoofddorp, the Netherlands) after 5 minutes supine rest. Finally, all subjects completed a questionnaire concerning their physical activity and health status, in which subjects were asked for medical history, medication usage, daily activity (hours of sport participating per week) and training status (walking-specific training history in the year preceding the walking march).

Table 1. Subject characteristics

Parameter	Sexagenarians (n=40)	Octogenarians (n=36)	P-value
Demographic characteristics			
Age (years)	60±1	81±2	**<0.01**
Height (cm)	172±10	168±9	0.08
Weight (kg)	81.5±15.5	68.3±11.3	**<0.01**
Body-mass index (kg/m^2)*	27.4±3.8	24.0±2.6	**<0.01**
Body fat (%)	34.0±5.8	28.2±6.9	**<0.01**
Lean body mass (kg)	54.0±10.9	49.0±9.1	**0.04**
Abdominal circumference (cm)	96.4±13.1	89.2±10.4	**0.01**
Men	102.3±12.8	94.4±8.6	**0.02**
Women	87.6±7.7	79.8±5.5	**<0.01**
Waist circumference (cm)	100.9±7.3	95.4±7.1	**<0.01**
Waist-Hip ratio	0.96±0.09	0.94±0.09	0.38
Health status			
Physical activity			
Exercise (h/week)*	3.1±2.9	4.8±4.5	0.29
Training status (km/year)*	501±423	990±723	**<0.01**
Mean arterial pressure (mmHg)	102±12	103±12	0.62
Rest heart rate (bpm)	67±12	67±16	0.95
Pathology			
Cardiovascular diseases (n(%))	6 (15.0%)	4 (11.1%)	0.62
Hypertension (n(%))	15 (37.5%)	14 (38.9%)	0.89
Hypercholesterolemia (n(%))	9 (22.5%)	8 (22.2%)	0.83
Diabetes (n(%))	2 (5.0%)	1 (2.8%)	0.57
Medication			
Anti-hypertensive drugs (n(%))	13 (32.5%)	15 (41.7%)	0.41
Diuretics (n(%))	5 (12.5%)	3 (8.3%)	0.56
Statins (n(%))	9 (22.5%)	5 (13.9%)	0.33
Other CVD medication (n(%))	7 (17.5%)	7 (19.4)%	0.83
Insulin medication (n(%))	2 (5.0%)	1 (2.8%)	0.62

P-value refers to an unpaired Students t-test or Pearson's Chi-square test between sexagenarians and octogenarians. * Ln-transformation was applied as a non-Gaussian distribution was present.

Thermoregulation

Core body temperature (Tc). A portable telemetry system (CoreTemp™ system, HQ Inc, Palmetto, USA) was used to measure Tc, which is a safe and reliable method to examine Tc at rest and during exercise[21, 22]. Subjects ingested an individually calibrated telemetric temperature pill on the evening preceding the exercise bout, to avoid interaction with fluid ingestion during testing[23]. Baseline Tc was determined as the average of 3 consecutive measurements. This procedure was repeated every 5 km along the route. The highest value of these measurements was presented as peak Tc and increment in Tc (ΔTc) was calculated as peak Tc minus baseline Tc.

Heart rate (HR). Heart rate was measured simultaneously with Tc (*i.e.* 3 consecutive measurements every 5 km) using a 2-channel ECG chest band system (Polar Electro Oy, Kempele, Finland). Mean heart rate during exercise was calculated as the average heart rate, excluding the values measured directly before the start and after the finish. Exercise intensity was calculated by dividing the mean heart rate during exercise by the maximal predicted heart rate (208-0.7*age)[24].

Fluid balance

Fluid intake. All subjects received written and individual oral instructions regarding the registration of their fluid intake. During exercise subjects were allowed to drink *ad libitum*, while they registered the time (in blocks of 1 hour), amount (using standard sized cups (125 mL), cans (330 mL) and bottles (500 mL)), and type ('water', 'sports drink' or 'other') of their individual fluid intake in a diary. Directly post-exercise, the fluid intake diary was checked by the research team, and clarifications were provided by the subjects if necessary. The relative change in body mass (in %) between the measurement immediately before the start and directly after finishing was calculated.

Sweat rate. Sweat rate (mL/h) was calculated as a combination of body mass, fluid intake and urine output data using the following formula: sweat rate (mL/h)= (pre-exercise body mass – post exercise body mass + fluid intake – urine output)/exercise duration[25].

Blood sodium levels + plasma volume. Subjects were seated for 5 minutes after which a 2 mL blood sample was drawn from the antecubital vein. Blood samples were directly analyzed for plasma levels of sodium, hemoglobin and hematocrit (Rapidpoint® 400, Siemens Healthcare Diagnostics Inc., Tarrytown, New York, USA). Hyponatremia and hypernatremia were defined as a plasma sodium concentration of ≤135 and ≥145 mmol/L, respectively[26, 27]. Relative changes in plasma volume were calculated from blood hematocrit and hemoglobin concentrations using Dill and Costill's equation[28].

Urine analysis. To determine urine specific gravity, a 5 mL urine sample was provided by all subjects and directly analyzed (Clinitek Status® Analyzer, Siemens Healthcare Diagnostics

Inc., Tarrytown, New York, USA). A urine specific gravity of ≥1.020 g/mL is indicative for dehydration[25]. To determine the total amount of urine output, subjects were instructed to exclusively urinate into a specialized collecting bag (Roadbag/Ladybag, KETs GmbH, Köln, Germany) during the entire exercise bout. Bags were collected after every 5 km and weighted at the laboratory within 0.1 g accuracy (PT 1400, Sartorius AG, Göttingen, Germany).

Ambient conditions. During the experiment, dry bulb, wet bulb and globe temperatures were measured every 30 minutes using a portable climate monitoring device (Davis instruments Inc., Hayward, USA), which is positioned at the start/finish area. The wet bulb globe temperature index (WBGT) was calculated using the formula: WBGT= 0.1 (Tdry bulb) + 0.7 (Twet bulb) + 0.2 (Tglobe)[29].

Statistical analysis

All values are presented as mean±standard deviation (SD), unless indicated otherwise. Statistical analyses were performed using Statistical Package for Social Sciences 20.0 (IBM SPSS version 20.0, Armonk, New York, USA) and the level of significance was set at $p<0.05$. The Kolmogorov-Smirnov test was used to examine the normality of the data distribution. When data demonstrated a non-Gaussian distribution, Ln-transformation was applied. Comparisons of baseline measurements between groups were assessed using an unpaired Student's t-test. To assess differences in fluid balance between the sexagenarians and the octogenarians, an unpaired Students t-test was used. A linear mixed model analysis was used to determine whether changes in Tc and exercise intensity across the 30-km exercise differed between both groups. A two-way (2X2) repeated measures ANOVA was used to examine whether exercise altered plasma sodium, hemoglobin and hematocrit concentration ('exercise': pre *versus* post), and whether these changes differed between groups ('group': sexagenarians *versus* octogenarians). Finally, a Pearson's Chi-square test was applied to assess differences in the prevalence of dehydration, hypo- or hypernatremia and high urine specific gravities between groups. Results of the Pearson's Chi-square test were presented as relative risk (RR) and their 95% confidence intervals.

RESULTS

Three octogenarian subjects did not finish the 30 km walking exercise bout due to a fractured wrist and severe muscle pains and fatigue, and were excluded from further analysis. The average walking distance in the year prior to the march was 501±423 km and 990±723 km for the sexagenarians and octogenarians, respectively ($p<0.01$). Octogenarians had a lower height, body mass, body mass index (BMI), body fat, lean body mass and abdominal and waist circumference compared to sexagenarians (all $p<0.05$, Table 1), whilst no differences were found for mean arterial pressure and heart rate. No differences in prevalence of cardiovascular

diseases, hypertension, hypercholesterolemia and diabetes were reported, additionally we found no differences in medication usage (Table 1, all p>0.05).

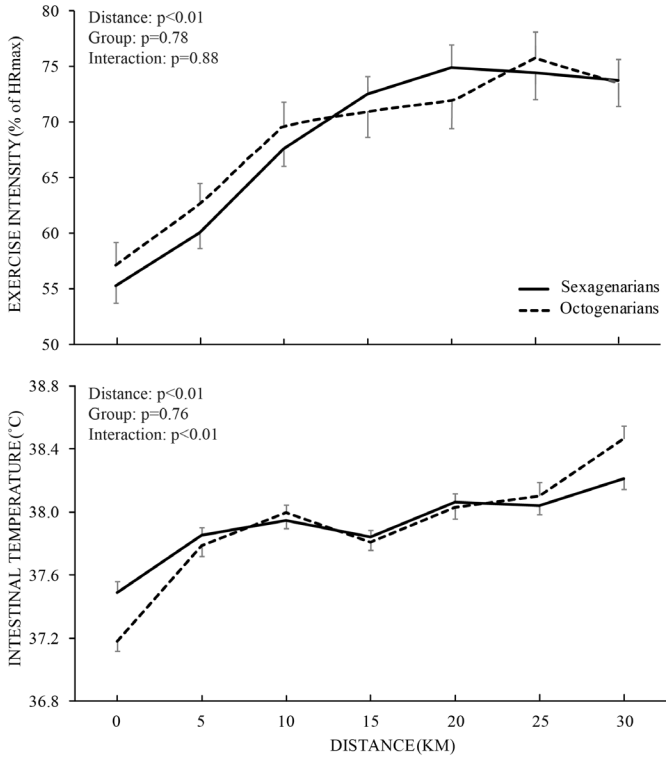


Figure 1. Time course of **(A)** exercise intensity (% of HRmax) and **(B)** core body temperature (°C) in sexagenarians (solid line) and octogenarians (dashed line). While, octogenarians demonstrated a larger increase in core body temperature compared to sexagenarians (p<0.01), no differences were observed in the change in exercise intensity between the groups (p=0.88). Data is presented as mean±SEM

Exercise characteristics

Relative humidity and wet bulb globe temperature (WBTG) were 95% and 12°C at the start of the exercise bout (i.e. 7.00 AM) and gradually changed to 43% and 24°C at 5.00 PM. Exercise duration and walking speed were comparable between both groups (Table 2). Exercise intensity increased during the exercise bout to a similar extent in both groups (p=0.88, Figure 1A).

Core body temperature

Baseline core body temperature (Tc) was 0.3°C lower in the octogenarians (37.2±0.3) compared to the sexagenarians (37.5±0.4, p<0.01). Tc increased significantly during exercise (p<0.01),

whilst the increase in Tc was significantly larger in octogenarians compared to sexagenarians (1.2±0.5°C *versus* 0.7±0.4°C, p<0.01). In contrary, maximum Tc tended to be higher in the octogenarians (38.4±0.4°C *versus* 38.2±0.3°C; p=0.09).

Fluid balance

Average fluid intake during exercise was lower for the octogenarians (251±97 mL/hour) compared to the sexagenarians (325±125 mL/hour, p=0.01, Figure 2A). Furthermore, octogenarian subjects demonstrated a significantly lower urine output compared to sexagenarians (p<0.01), and the sweat rate tended to be lower in the octogenarian group (p=0.07, Figure 2B-C). Post-exercise plasma volume, plasma sodium, hemoglobin and hematocrit concentration, and incidence of urine specific gravity levels ≥1.020 g/mL were not different between groups (all p>0.05, Table 2). Both groups demonstrated an exercise-induced increase in hemoglobin and hematocrit concentration (p<0.01), whilst the exercise-induced changes in plasma sodium concentration were not different in both groups (p>0.05). Both groups demonstrated a decrease in body mass after exercise. The relative decrease in body mass was significantly larger for the octogenarians (p=0.04, Figure 2D), whilst no differences between groups were observed in dehydration status (Table 2).

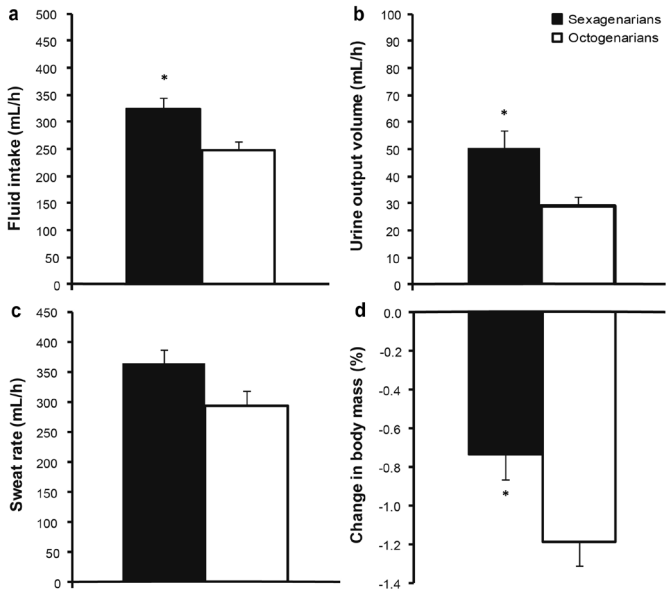


Figure 2. Fluid balance parameters **(A)** Fluid intake, **(B)** urine output, **(C)** sweat rate and **(D)** body mass loss presented for both sexagenarians (black bars) and octogenarians (white bars). Sexagenarians demonstrated a higher fluid intake and sweat rate and a lower body mass loss. Data were presented as mean±SEM. * Represents a significant difference between

Table 2. Exercise characteristics and fluid balance presented per age group

Parameter	Sexagenarians	Octogenarians	P-value
Exercise characteristics			
Exercise duration (hh:mm)	7:24±0:58	7:45±0:57	0.13
Average walking speed (km/h)*	4.1±0.5	3.9±0.5	0.13
Average HR (bpm)	116±15	106±16	**<0.01**
Average exercise intensity (% of HR max)	70±9	70±11	0.97
Baseline Tc (°C)	37.5±0.4	37.2±0.3	**<0.01**
ΔTc (°C)	0.7±0.4	1.2±0.5	**<0.01**
Peak Tc (°C)	38.2±0.3	38.4±0.4	0.09
Fluid balance parameters			
Fluid intake (mL/h)	325±125	251±97	**0.01**
Water (%)	62.3±26.1	51.7±30.9	0.11
Other (%)	37.7±26.1	48.3±30.9	0.11
Sweat rate (mL/h)	364±148	294±150	0.07
Urine output (mL/h)*	52±40	28±22	**<0.01**
Urine specific gravity ≥1.020 g/mL (n(%))	33 (80.5%)	31 (75.6%)	0.88
Sodium concentration (mmol/L)*	142.0±3.3	141.1±4.5	0.33
Prevalence of hyponatremia (n(%))	1 (2.5%)	3 (7.7%)	0.24
Prevalence of hypernatremia (n(%))	6 (15.0%)	5 (12.8%)	0.94
Hemoglobin (mmol/L)	9.7±0.8	9.4±0.8	0.15
Hematocrit (L/L)*	45.8±3.8	44.7±3.7	0.22
Plasma volume change (%)*	-3.9±5.3	-3.5±6.2	0.19
Body mass change (kg)	-0.7±0.8	-0.8±0.7	0.36
Body mass change (%)	-0.7±0.9	-1.2±1.0	**0.04**
Prevalence of dehydration (≥2% body mass loss)	2 (5.0%)	6 (15.4%)	0.10

Tc= core body temperature; HR= heart rate. P-value refers to an unpaired Students t-test or Pearson's Chi-square test between sexagenarians and octogenarians. * Ln-transformation was applied as a non-Gaussian distribution was present.

DISCUSSION

The purpose of our study was to compare thermoregulatory responses and fluid balance during prolonged, moderate-intensity exercise between sexagenarians and octogenarians. We found that octogenarians demonstrate a lower baseline Tc and a larger rise in Tc during prolonged walking exercise compared to sexagenarians. As workload and exercise intensity were similar between groups, these results may suggest that the thermoregulatory control declines with advanced age. Moreover, octogenarians reported a lower fluid intake and urine output in

combination with a larger loss of body mass compared to sexagenarians, whilst other fluid balance parameters were not different between groups. Thus, in moderate ambient conditions and with ad libitum fluid intake the fluid balance regulation of both groups was adequate to avoid development of sodium disturbances or severe dehydration.

In accordance with literature, baseline Tc was 0.3°C lower in octogenarians compared to sexagenarians[30]. The most likely explanations for this observation is the smaller muscle mass and, concomitant, lower metabolic heat production in the oldest group[8, 30]. Furthermore, higher age was associated with a significantly higher exercise-induced increase in Since exercise characteristics were similar between groups (*i.e.* duration and intensity), we propose 3 possible explanations for the higher increase in Tc in the octogenarians. First, the sweat rate tended to be lower in aged subjects due to a decrease in individual sweat gland output as well as a reduced number of activated sweat glands by heat[8, 31]. Presumably, when aging progresses the sweat rate response is further impaired, which may result in a larger increment of Tc during prolonged exercise. Second, the initiation of the thermoregulatory responses to heat exposure occurs by altered signaling from thermosensors located both in the periphery and the core of the body[8]. With advanced age, there is a higher peripheral fiber loss and a lower conduction velocity of the nervous system, which may delay vascular responses to heat. This results in a decreased peripheral thermal sensation, a delayed response to heat exposure[8, 10], and subsequently a larger exercise-induced increase in Tc. A third possible mechanism relates to the attenuated cutaneous vasodilator response to heat in aged people[32, 33]. Both, a delay in the initiation of the vasodilatory as well as a decreased vasodilatory capacity of the skin are known to limit heat dissipation[32, 33]. The above mentioned factors limit the human skin to lose heat, thereby causing Tc to rise and potentially increase the risk to develop heat related illnesses. In parallel, a trend (p=0.09) for differences in maximum Tc between octogenarians and sexagenarians was observed. Whilst the present study was performed in moderate ambient conditions and maximum Tc of both groups was within the normal physiological range, differences in maximum Tc may be more pronounced under hot and humid ambient conditions. In summary, our data may suggest a progressive age-related impairment of thermoregulatory responses, with larger exercise-induced Tc increases in octogenarians compared to sexagenarians.

Octogenarians demonstrated a larger relative body mass loss compared to sexagenarians, suggesting a deficiency in fluid intake and/or a higher fluid loss. Indeed, the self-reported fluid balance diaries demonstrated that octogenarians consumed 74 mL/h less fluid compared to sexagenarians. This observation is in line with Phillips *et al.*[14] who demonstrated that older subjects (67-75 years) reported a lower thirst sensitivity compared to young controls (20-31 years) after a 24-h period of water deprivation. The blunted thirst response in older humans was later confirmed by others, who compared young and old subjects[18, 34]. Next to the differences in fluid intake, the octogenarians also reported a lower urine output. It may be assumed that there

is a causal relationship between the lower fluid intake and the lower urine output. The lower total body water content may enhance water reabsorption in the kidneys and hence reduce the urine production. Despite the lower fluid intake and urine output, no differences were observed between groups for changes in body mass, blood sodium concentration and plasma volume. These findings suggest that octogenarians and sexagenarians are both capable to regulate their fluid balance appropriately during prolonged walking exercise.

Clinical relevance. Health problems like heat stroke, dehydration and hyponatremia are frequently reported during prolonged exercise. Although octogenarians reported a larger Tc increase and lower fluid intake compared to sexagenarians, medical problems did not occur in both groups. Risk assessment must be revised when these vulnerable groups perform exercise in hot and humid ambient conditions. Particularly since recent fluid intake guidelines advice athletes to drink according their thirst sensation to avoid dehydration[35, 36] and previous studies noted a decreased thirst sensitivity in elderly[13, 14, 34]. For that reason, the "drink according to thirst" regimen may be inappropriate in the older individuals under severe ambient conditions. Therefore, it is recommended that future fluid replacement guidelines should include specific information on rehydration in elderly.

This study includes two unique groups of advanced aged subjects that performed prolonged walking exercise. However, one might argue that these individuals do not represent the general advanced age population, since these subjects have a highly active lifestyle. Consequently, there may be selection bias in the current study. Fact is that medical advances stimulate healthy aging and subsequent longevity in modern society. As a direct consequence, physical activity is increasingly popular in this population, which is reinforced by the growing number of older humans that participate in endurance exercise events. Therefore, insight into the physiological responses of these individuals is necessary for a safe exercise environment with appropriate recommendations. Secondly, sexagenarians reported a significantly higher BMI and lean body mass compared to octogenarians. As these characteristics increase the risk for fluid disturbances and the development of dehydration in sexagenarians[37], we may have underestimated the differences in thermoregulatory and fluid balance responses between both groups. A third limitation of this study is the used estimation of the maximum heart rate to calculate the exercise intensity. Since we used the formula by Tanaka *et al*.[24], a widely used method to calculate the predicted maximum heart rate, we believe that the consecutively calculated exercise intensities properly reflect the real exercise intensities. Finally, clothing was not reported during the study, while it plays an important role in the capacity to dissipate heat. However, the majority of the subjects wore normal walking clothes (t-shirt+shorts), and therefore, we strongly believe that clothing did not influence our results.

In conclusion, octogenarians demonstrate an impaired thermoregulatory control compared to sexagenarians during prolonged moderate-intensity exercise under moderate ambient conditions. In contrast, fluid balance was well controlled in both groups and did not deteriorate with aging. This enabled sexagenarians as well as the octogenarians to successfully complete a 30 km walking march without heat or fluid related health problems.

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Chapter 10

Impact of Acute versus Repetitive Moderate Intensity Endurance Exercise on Kidney Injury Markers

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Physiological Reports

Impact of acute versus repetitive endurance exercise on kidney injury markers

60 Participants (29-78 years, 50% male) completed a prolonged walking exercise on three consecutive days

Exercise intensity

30-50 KM

	Baseline	Single day	Repetitive days
Dehydration - Body mass loss (%)	-	0.9±1.2% ↑	0.4±0.7% ↓
Kidney Function - Glomerular filtration rate (mL/min)	89.3±11.6	79.4±16.3 ↓	89.8±13.9 Not different
Kidney injury - uNGAL (pg/ng Cystatin C)	2.2 (1.3-3.2)	2.7 (1.8-4.0) ↑	2.5 (1.8-3.8) ↑
Kidney injury - uKim1 (pg/ng Cystatin C)	0.6 (0.4-0.9)	0.6 (0.4-1.0) Not different	0.6 (0.4-0.8) Not different

Prolonged exercise did result in kidney injury, while there is no cumulative effect of repetitive exercise

ABSTRACT

Exercise may lead to kidney injury through several mechanisms. Urinary Kidney Injury Molecule-1 (uKIM1) and Neutrophil Gelatinase-Associated Lipocalin (uNGAL) are known biomarkers for acute kidney injury, but their response to repetitive exercise remains unknown. We examined the effects of a single *versus* repetitive bouts of exercise on markers for kidney injury in a middle-aged population. Sixty subjects (aged 29-78 years, 50% male) were included and walked 30, 40 or 50 km for three consecutive days. At baseline and after exercise day 1 and 3, a urine sample was collected to determine uNGAL and uKIM1. Furthermore, urinary cystatin C, creatinine and osmolality were used to correct for dehydration-related changes in urinary concentration. Baseline uNGAL was 9.2(5.2-14.7) ng/mL and increased to 20.7(11.0-37.2) ng/mL and 14.2(8.0-26.3) ng/mL after day 1 and day 3, respectively (p-values≤0.001). Baseline uKIM1 concentration was 2.6(1.4-6.0) ng/mL and increased to 5.2(2.4-9.1) ng/mL (p=0.002) after day 1, whereas uKIM1 was not different from baseline at day 3 (2.9(1.4-6.4) ng/mL (p=0.52)). Furthermore, both uNGAL and uKIM1 levels were higher after day 1 compared to day 3 (p<0.01). When corrected for urinary cystatin C, creatinine and osmolality, uNGAL demonstrated a similar response compared to the uncorrected data, whereas differences in uKIM1 between baseline, day 1 and day 3 (p_{time}=0.63) were no longer observed for cystatin C and creatinine corrected data. A single bout of prolonged exercise significantly increased uNGAL concentration, whereas no changes in uKIM1 were found. Repetitive bouts of exercise show that there is no cumulative effect of kidney injury markers.

INTRODUCTION

Exercise is a major challenge to whole-body homeostasis provoking widespread perturbations in cells, tissues, and organs that are caused by or are a response to the increased metabolic activity of contracting skeletal muscles[1]. Maintaining an adequate fluid and electrolyte homeostasis is a key role of the kidneys, which is affected by endurance exercise due to exercise-induced dehydration[2]. The exercise-induced redistribution of blood to active body parts[3], causes a decreased renal perfusion, an increased glomerular permeability and a decreased filtration ratio[4-7]. These and other alterations may lead to acute, but transient, kidney injury. Moreover, animal studies demonstrated an increased number of apoptotic cells in distal tubules after cessation of exercise, which is indicative for kidney injury[8].

Traditional markers for assessing renal function are the estimated glomerular filtration rate (eGFR) and serum creatinine concentration[9, 10]. These markers are late stage markers for a decreased kidney function, primarily defined as glomerular filtration rate (GFR), and less well-suited for the detection of initial kidney injury[11]. Over the past years, a number of new biomarkers have been identified to detect acute kidney injury in an early stage, of which Kidney Injury Molecule-1 (KIM1) and Neutrophil gelatinase-associated lipocalin (NGAL) are best known[10, 12, 13]. Previous studies suggested that a single bout of short-term high intensity exercise[14] or completing a (ultra)marathon[15-17] increases urinary excretion of KIM1 (uKIM1) and NGAL (uNGAL). It has been suggested that exercise intensity is the most important factor that impacts on exercise-induced kidney stress. Literature found a reduced GFR at exercise intensities of 60% and 80%, whereas no difference in pre- and post-exercise GFR was found at lower exercise intensities[18]. Therefore, the question raises whether uKIM1 and uNGAL increase after prolonged walking exercise (~70% intensity) as well, which is a more accessible type of exercise for the whole population. In addition, the interpretation of the findings of previous studies is difficult as uKIM1 and uNGAL were only corrected for urinary creatinine concentration, which might be influenced by exercise and in particular exercise-induced muscle breakdown[14]. Therefore, it is hard to distinguish whether observed changes are the effect of exercise or due to an increased urine concentration because of dehydration. Additionally, it is unknown whether the kidney's response to repetitive exercise is similar to that of a single bout of prolonged exercise. Since training programs, especially for endurance athletes, consists of exercise bouts on consecutive days, it is relevant to know whether renal function might be affected in a cumulative way.

Therefore, the aim of this study was to examine the effect of a single *versus* repetitive bouts of prolonged walking exercise in healthy adults on markers for kidney injury in order to establish the physiological responses of the kidneys to prolonged and repeated endurance exercise. To account for exercise-induced changes in urinary concentration, uNGAL and

uKIM1 will be corrected for cystatin C, creatinine and osmolality. We hypothesized that a single bout of endurance exercise causes an increase in biomarkers for kidney injury, whereas a cumulative kidney injury biomarker response will be observed after three consecutive days of prolonged exercise.

METHODS

Subjects

A total of 60 subjects (aged 29 – 78 years, 30 men and 30 women) participated in this prospective intervention study. All subjects participated in the largest walking march in the world (*i.e.* the International Nijmegen Marches), in which subjects walked at a self-selected pace for 30 km (n=13), 40 km (n=37) or 50 km (n=10) on consecutive days. Subjects with self-reported kidney disease were excluded from study participation. The study was approved by the Medical Ethical Committee of the Radboud University Medical Center, and all subjects gave written informed consent prior to participation in the study.

Experimental design

Data was collected at baseline (12 to 36 hours before the start of the walking event) and directly after walking day 1 and 3. Baseline measurements were conducted in controlled laboratory conditions and consisted of assessment of subject characteristics (*i.e.* age, body mass index, fat percentage, blood pressure and habitual physical activity levels) and collection of a blood and urine sample for the assessment of fluid balance, eGFR and kidney injury parameters. Prior to the onset of exercise at day 1 and 3, body mass was assessed. Subsequently, subjects were instructed to walk at a self-selected pace. Heart rate was monitored every 5 km during the first walking day, and subjects were asked to register their fluid intake during the whole exercise bout. Directly after the finish of day 1 and 3, body mass was measured and a blood and urine sample was taken.

Measurements

Subject characteristics. At baseline, body mass (SECA 888 scale, Hamburg, Germany) and body height were measured, which were used to calculate the body mass index (BMI). Furthermore, body fat percentage was calculated using a four-point (biceps, triceps, sub-scapular and sub-iliac) skinfold thickness measurement[19]. Thereafter, blood pressure and heart rate were measured twice using an automated sphygmomanometer (M5-1 intellisense, Omron Healthcare, Hoofddorp, the Netherlands) after 5 minutes of supine rest. Finally, all subjects completed a questionnaire regarding their habitual physical activity levels, including the hours of exercise per week, and the walking specific training history in the year prior to the walking march.

Blood sample. A venous blood sample was taken to determine plasma levels of sodium, hematocrit and hemoglobin (Rapidpoint® 400, Siemens Health Care Diagnostics Inc., Tarrytown, New York, USA) at baseline and directly after exercise cessation. Using changes in plasma hematocrit and hemoglobin concentration the relative plasma volume change (in %) was calculated according to the formula by Dill and Costill[20]. Hypo- and hypernatremia were defined as plasma sodium concentrations of ≤135 and ≥145 mmol/L, respectively[21, 22]. Furthermore, serum creatinine was measured at baseline and directly after completion of the exercise on day 1 and day 3. The estimated glomerular filtration ratio (eGFR) was calculated to assess baseline kidney function using the CKD-EPI creatinine equation, which contained the serum creatinine concentration as well as subject's age, gender and ethnicity[23]. Furthermore, plasma interleukine-6 (IL-6) and tumor necrosis factor-α (TNF-α) were measured to examine differences in inflammatory response between baseline and post-exercise.

Urine sample. At baseline and directly after exercise cessation, all subjects provided a urine sample to examine kidney injury and fluid balance. Urinary cystatin C concentration was measured using the nephelometric method (Behring Nephelometer II, Siemens Healthcare, Den Haag, The Netherlands). Furthermore, the urinary creatinine concentration was measured using the enzymatic method (Cobas C6000, Roche Diagnostics, Indianapolis, USA). Urine osmolality was examined using an osmometer (Advanced Model 3320 Micro-Osmometer, Osmometer, Advanced Instruments, Norwood, USA). Urine specific gravity and urine proteinuria was assessed using a dip-stick method (Clinitek Status® Analyzer, Siemens Healthcare Diagnostics Inc., Tarrytown, New York, USA). Proteinuria was defined using a categorical scale: negative (0 g/L), trace (0.15 g/L), + (0.3 g/L) or ++ (1 g/L).

In order to determine kidney injury in response to exercise, we measured urinary concentrations of KIM1 and NGAL (both monomeric and dimeric) in duplicate using the previously described sandwich ELISA assay (E-EL-H0186 and E-EL-H0096, Elabscience Biotechnology, Wuhan, China)[24, 25]. Furthermore, uKIM1 and uNGAL concentrations were corrected for urinary cystatin C, creatinine and osmolality. The corrected uKIM1 and uNGAL data were calculated by dividing the individual uncorrected data by the corresponding cystatine C and creatinine levels and the urine osmolality. Furthermore, acute kidney injury was defined based on the Acute Kidney Injury Network (AKIN) criteria[26]. Stage 1 was defined as a 1.5 to 2-fold or 26.4 µmol/L increase in serum creatinine concentration from baseline to peak value on either day 1 or day 3, whereas stage 2 was defined as a 2 to 3-fold increase in serum creatinine concentration[26].

Fluid balance. All subjects received written and individual oral instructions about the registration of their fluid intake in a diary. Subjects were allowed to drink *ad libitum*, as long as they registered the time (in blocks of 1 hour), amount (standardized sized cups, bottles, etc.) and type (water, sports drink, other) of their individual fluid intake 12 hours prior to the start

and throughout the walking exercise. Furthermore, body mass was measured prior to and directly after finishing the walking march. Relative change in body mass (in %) between both measurements was calculated. We defined dehydration as a relative body mass loss ≥2%[27].

Exercise intensity. Heart rate was measured at every 5 kilometer milestone during the first walking day using a 2-channel chest band (Polar Electro, Oy, Kempele, Finland), which was instrumented to the subjects prior to the start. The average of at least 3 consecutive heart rate measurements (~15 seconds) was taken at every 5 km point while walking. Measurements prior to the start and directly after finishing were excluded for the calculation of the mean heart rate during exercise. Exercise intensity (%) was calculated by dividing the mean heart rate by the predicted maximal heart rate according to Tanaka's formula (HRmax=208-0.7*age)[28].

Ambient conditions. The dry bulb, wet bulb and globe temperature, as well as the relative humidity were measured every 30 minutes throughout the experiment using a portable climate monitoring device (Davis Instruments inc., Hayward, U.S.A), which was located at the start/finish area. Based on the above mentioned temperatures, the wet bulb globe temperature (WBGT) was calculated using the formula: $WBGT=0.1(T_{dry\ bulb}) + 0.7(T_{wet\ bulb}) + 0.2(T_{globe})$[29].

Statistical analysis

Normally distributed data were presented as mean ± standard deviation (SD), whilst non-Gaussian distributed data were presented as median (interquartile range). The statistical analysis were conducted using the Statistical Package for Social Sciences (SPSS version 20, Armonk, NY, USA), in which the level of significance was set at $p<0.05$. Data was checked for normality using the Shapiro-Wilk test. In case of non-Gaussian distributed data, the statistical analysis were performed using the non-parametric equivalents. The effect of acute exercise on kidney injury and fluid balance was examined using a paired Students T-test or a Wilcoxon signed rank test. A repeated measures ANOVA was used to assess differences in kidney injury and fluid balance over time, which was used to determine the effects of repetitive exercise. Subsequently, a post-hoc Bonferroni test was used to determine differences between individual experimental days. In case of Non-Gaussian distributed data a Friedman test was used to assess differences in kidney injury over time, followed by a Wilcoxon signed rank test to determine differences between individual days. A Kruskal-Wallis test was used to determine sex differences in kidney response to acute an repetitive bouts of exercise. A Chi-square test was used to determine differences in the prevalence of dehydration, hypo- or hypernatremia, high urine specific gravities and proteinuria.

Table 1. Baseline subject characteristics (n=60)

Parameter	Value
Sex (male:female)	30 : 30
Age (years)	56±10
Body length (m)	173±8
Body mass (kg)	75.4±13.8
BMI (kg/m^2)	24.9±3.0
Fat percentage (%)	31.2±5.5
Systolic blood pressure (mmHg)	138±20
Diastolic blood pressure (mmHg)	85±11
Resting heart rate (bpm)	63±7
eGFR (mL/min/1.73 m^2)	89.3±11.6
Activity score (au)	6,965±3,462
MET min a day (MET min)	1,027±502

Subject characteristics for the total group. Data were presented as mean±SD. MET= Metabolic equivalent of task, bpm= beats per minute, au= arbitrary unit.

RESULTS

Subject and Exercise characteristics. At baseline the average eGFR was 89.3±11.6 mL/min/1.73 m^2 (Table 1). All subjects successfully completed the walking exercise bouts. An overview of baseline characteristics is shown in Table 1. Exercise duration and walking speed did not differ between both experimental days (Table 2). Moreover, subjects completed the exercise bout at 71±9% of their predicted maximal heart rate, and walked for 8-9 hours with an average walking speed of 4.8 km/h. WBGT ranged between 13°C (04.00 AM) and 24°C (17.00 PM), and average WBGT was higher on day 1 *versus* 3 (21.1°C versus 17.6°C, p<0.001), whereas the relative humidity did not differ between both days (p=0.08). Elevated plasma IL-6 and TNF-α levels were found after both exercise day 1 and day 3 compared to baseline (all p-values <0.05), whereas IL-6 and TNF-α were higher after day 1 compared to day 3 (p<0.001, Table 2). Additionally, no correlation was found between inflammatory markers and corrected as well as uncorrected uNGAL concentrations (all p-values >0.05) after day 1 and day 3.

Fluid balance. Fluid balance data are shown in Table 2. A significant decrease in body mass was found after both, day 1 and day 3 (both p-values <0.001). Body mass losses were significantly larger at day 1 (-0.9±1.2%) compared to day 3 (-0.4±0.7%, p=0.005). In line with this, 12 subjects (20%) were considered dehydrated after day 1 (body mass loss ≥2%), while none of the subjects were dehydrated after day 3 (p<0.001). Furthermore, we demonstrated an exercise-induced increase in urine specific gravity on both, day 1 and day 3 (p=0.005 and p=0.025 respectively),

with a comparable incidence of urine specific gravity levels ≥1.020 g/mL on day 1 compared to day 3 (p=0.54). Post-exercise plasma hemoglobin and hematocrit levels were increased compared to baseline (both p-values <0.001), in which hemoglobin and hematocrit levels were higher after day 1 compared to day 3 (both p-values <0.001). Furthermore, plasma sodium concentration and the prevalence of hypernatremia were increased after day 1, but not after day 3 (both p-values <0.001).

Kidney injury – uncorrected data. Absolute median uNGAL concentration was 9.2 (5.2 - 14.7) ng/mL at baseline and increased post-exercise to 20.7 (11.0 - 37.2) ng/mL and 14.2 (8.0 – 26.3) ng/mL on day 1 and day 3, respectively (both p-values ≤0.001, Figure 1A). Uncorrected uNGAL levels after day 1 were significantly higher compared to day 3 (p<0.001). For uncorrected uKIM1, median baseline concentration was 2.6 (1.4 – 6.0) ng/mL and increased to 5.2 (2.4 – 9.1) ng/mL (p=0.002, Figure 2A) after exercise day 1 and did not differ from baseline after day 3 (2.9 (1.4 – 6.4) ng/mL (p=0.52)).Furthermore, uncorrected uKIM1 concentration after day 1 was higher compared to day 3 (p=0.003).

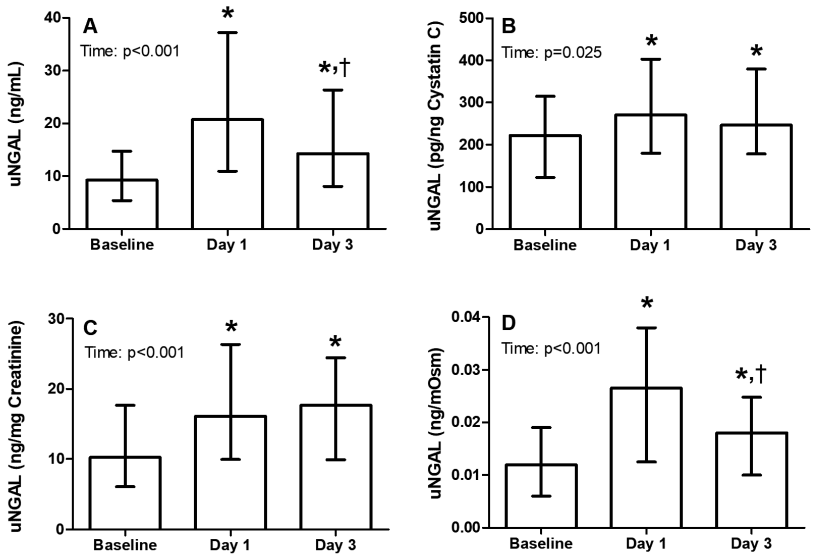

Figure 1. Effect of acute and repetitive bouts of prolonged exercise on uNGAL concentration (n=60). The uncorrected data are presented **(A)**, as well as after correction for cystatin C **(B)**, creatinine **(C)** and osmolality **(D)**. A significant increase in NGAL concentration compared to baseline was found after day 1 and 3. Data were presented as median with interquartile range. * Represents a significant difference from baseline and † represents a difference from day 1.

Table 2. Exercise characteristics and fluid balance parameters at baseline and after day 1 and day 3

	Baseline	Day 1	Day 3	P-value
Exercise characteristics				
Exercise duration (hh:mm)	-	8:10±1:59	8:28±1:53	0.29
Average HR (bpm)	-	112±14	-	-
Exercise intensity (% of HR max)	-	70.8±8.9	-	-
Walking speed (km/h)	-	4.8±0.7	4.7±0.7	0.22
WBGT (°C)	-	21.1±1.5	17.6±2.5†	**<0.001**
Relative humidity (%)	-	71.4±14.9	67.5±19.6	0.08
Fluid balance				
Fluid intake (mL/h)	-	309±121	300±133	0.48
Water (%)	-	50.7±22.2	48.1±21.0	0.26
Other (%)	-	49.3±22.2	51.9±21.0	0.26
Urine osmolality (mOsm/mL)§	890 (589-1113)	998 (719-1159)	945 (649-1264)	0.36
Hemoglobin (mmol/L)	15.2±1.4	15.5±1.5*	14.8±1.5*†	**<0.001**
Hematocrit (L/L)	44.8±4.2	45.6±4.4*	43.5±4.5*†	**<0.001**
Plasma volume change (%)	-	-2.9±8.5	5.8±10.7†	**<0.001**
Plasma sodium (mmol/L)	141.2±1.6	143.4±2.4*	141.4±2.0†	**<0.001**
Prevalence of hyponatremia (n(%))	0 (0%)	0 (0%)	0 (0%)	1.00
Prevalence of hypernatremia (n(%))	1 (1.7%)	18 (30%)*	2 (3.3%)†	**<0.001**
Body mass change (kg)	-	-0.8±1.0	-0.4±0.5†	**0.001**
Body mass change (%)	-	-0.9±1.2	-0.4±0.7†	**0.005**
Dehydration; ≥2% body mass loss (n(%))	-	12 (20%)	0 (0%)†	**<0.001**
Inflammation				
Plasma IL-6 (pg/mL)	0.5 (0.3-0.6)	5.2(3.4-8.4)*	2.0(1.5-3.5)*†	**<0.001**
TNF-α (pg/mL)	1.53 (1.3-1.8)	1.6 (1.4-1.9)*	1.5 (1.3-1.7)*†	**<0.001**

Exercise characteristics and fluid balance at baseline, after exercise day 1 and day 3. Data were presented as mean±SD or median (IQR). P-values represents the results of the one-way ANOVA or non-parametric alternative. *Significantly different from baseline. † Significantly different from day 1. § A non-parametric alternative was used.

Kidney injury – cystatin C corrected data. Urinary cystatin C concentration was significantly elevated after day 1 (0.09 (0.05-0.12 mg/L)) compared to baseline (0.05 (0.03-0.08 mg/L); p<0.001), but this effect disappeared at day 3 (0.06 (0.03-0.09 mg/L); p=0.28). Cystatin C corrected uNGAL concentration at baseline was 222 (134-316) pg/ng cystatin C, and increased to 271 (180-403) pg/ng cystatin C (p=0.002) and 247 (178-379) pg/ng cystatin C (p=0.010) after

day 1 and day 3, respectively (p_{time}=0.025, Figure 1B). Post-exercise uNGAL concentration did not differ between day 1 and day 3 (p=0.75). A total of 39 (65%) subjects demonstrated elevated uNGAL levels after day 1 and/or day 3, but no differences between day 1 and day 3 were observed (p=1.00). uKIM1 corrected for urinary cystatin C concentration showed no differences across days (p_{time}=0.63, Figure 2B).

Kidney injury – creatinine corrected data. Urinary creatinine concentration was significantly elevated after day 1 (1.31 (0.89-2.02 mg/mL)) compared to baseline (0.92 (0.61-1.50 mg/mL); p<0.001), but this effect disappeared at day 3 (0.77 (0.52-1.48 mg/mL); p=0.80). Creatinine corrected uNGAL concentration at baseline was 10.3 (6.1-17.7) ng/mg creatinine, and increased to 16.1 (10.0-26.4) ng/mg creatinine (p<0.001) and 17.7 (9.9-24.4) ng/mg creatinine (p<0.001) after day 1 and day 3, respectively (p_{time}<0.001, Figure 1C). Post-exercise creatinine corrected uNGAL concentration did not differ between day 1 and day 3 (p=0.48). uKIM1 corrected for urinary creatinine concentration showed no differences across days (p_{time}=0.27, Figure 2C).

Kidney injury – osmolality corrected data. Baseline urine osmolality was 890 (589-1113 mOsm/mL) and demonstrated a non-significant (p=0.36) increase after completion of the exercise bout on both day 1 (998 (719-1159 mOsm/mL)) and day 3 (945 (649-1264 mOsm/mL)). Baseline osmolality corrected uNGAL concentration was 0.012 ng/mOsm, with increased levels after day 1 (0.027 (0.013-0.038) ng/mOsm) and after day 3 (0.18 (0.010-0.025) ng/mOsm) (p_{time}<0.001, Figure 1D). Furthermore, osmolality corrected uNGAL levels after day 1 were significantly higher compared to day 3 (p<0.001). Osmolality corrected uKIM1 was 0.004 (0.002-0.006 ng/mOsm) at baseline and increased to 0.006 (0.003-0.009 ng/mOsm) after exercise day 1 (p=0.003) and did not differ from baseline after day 3 (0.003 (0.002-0.005) ng/mOsm, p=0.49, Figure 2D). Furthermore, osmolality corrected uKIM1 concentration after day 1 was higher compared to day 3 (p=0.002).

Kidney injury – AKIN criteria and eGFR. Serum creatinine concentration was significantly elevated after day 1 (86.9±22.8 µmol/L) compared to baseline (75.3±13.7 µmol/L; p<0.001), but this effect disappeared at day 3 (75.6±14.9 µmol/L; p=0.81). Moreover, 10% of the subjects (n=6) developed stage 1 acute kidney injury by AKIN criteria after day 1, whereas none of the subjects developed stage 1 acute kidney injury after day 3. Additionally, none of the subjects developed stage 2 kidney injury. At baseline the average eGFR was 89.3±11.6 mL/min/1.73 m^2 which decreased after day 1 to 79.4±16.3 mL/min/1.73 m^2 (p<0.001), but no difference was found after day 3 (89.8±13.9 mL/min/1.73 m^2; p=0.70). Furthermore, n=2 subjects were traced with proteinuria at baseline (Table 3), n=8 subjects after day 1 (range: 0.15-1 g/L) and n=5 after day 3 (range: 0.15-0.30 g/L, p=0.39).

Table 3. Renal injury markers at baseline and after day 1 and day 3

	Baseline	Day 1	Day 3	P-value
Urine Cystatin C (mg/L)§	0.05 (0.03-0.08)	0.09 (0.05-0.12)*	0.06 (0.03-0.09)	**<0.001**
Urine Creatinine (mmol/L)§	8.1 (5.4-13.3)	11.6 (7.9-17.9)*	6.8 (4.6-13.1)†	**<0.001**
Serum Creatinine (µmol/L)	75.3±13.7	86.9±22.8*	75.5±15.0†	**<0.001**
Proteinuria				0.39
Negative (0 g/L) (n(%))	58 (96.7%)	52 (86.7%)	55 (91.7%)	
Trace (0.15 g/L) (n(%))	2 (3.3%)	3 (5%)	2 (3.3%)	
+ (0.3 g/L) (n(%))	0 (0%)	4 (6.7%)	3 (5%)	
++ (1 g/L) (n(%))	0 (0%)	1 (1.7%)	0 (0%)	

Renal injury markers at baseline, after exercise day 1 and day 3. Data were presented as median (IQR). * Significantly different from baseline. † Significantly different from day 1. § A non-parametric alternative was used.

Subject and exercise characteristics associated with kidney injury. The increase in uncorrected and osmolality corrected uNGAL and uKIM1 in response to acute exercise was higher for men compared to women (all p-values <0.05), while no differences were found for cystatin C and creatinine corrected data. After repetitive bouts of prolonged exercise no differences were found between men and women (all p-values >0.05), except for a lower creatinine corrected uNGAL concentration for men compared to women (p=0.005). Furthermore, for both men and women no correlation was found between age and uNGAL/uKIM1 levels (uncorrected and corrected) after both acute and prolonged exercise. Additionally, the physical activity level of the subjects was not related to kidney injury outcomes. No relationship was found between kidney injury and walking distance or exercise duration after both acute and repetitive bouts of prolonged exercise. Furthermore, the average exercise intensity during a single bout of prolonged exercise was not related to the uKIM1 concentration and creatinine and cystatin C corrected uNGAL, whereas a higher exercise intensity is related to higher uncorrected and urine osmolality corrected uNGAL levels (both R^2=0.27, p=0.043).

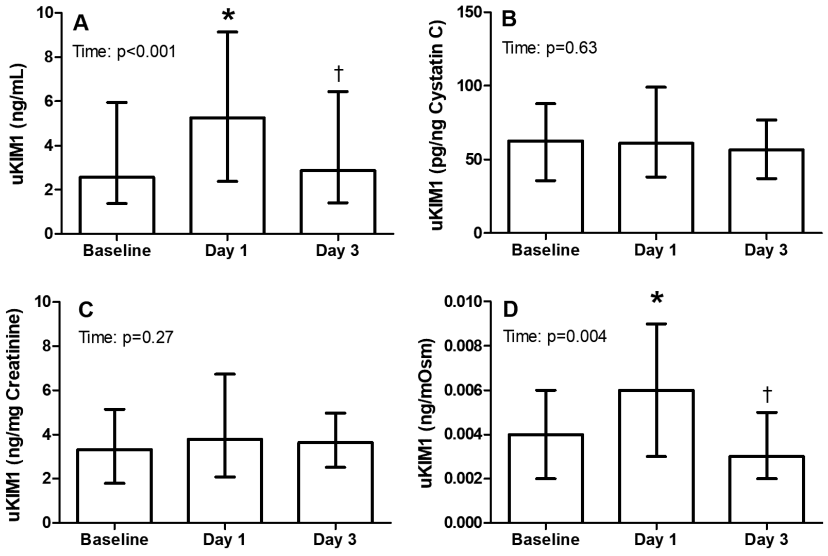

Figure 2. Effect of acute and repetitive bouts of prolonged exercise on uKIM1 concentration (n=60). The uncorrected data are presented **(A)**, as well as after correction for cystatin C **(B)**, creatinine **(C)** and osmolality **(D)**. Increased uKIM1 levels after day 1 compared to baseline were found for the uncorrected and osmolality corrected data, whereas no differences in uKIM1 between days were found after cystatin C and creatinine correction. Data were presented as median with interquartile range. * Represents a significant difference from baseline and † represents a difference from day 1.

DISCUSSION

The purpose of this study was to examine the effects of acute as well as repetitive bouts of prolonged exercise on markers of kidney injury. When corrected for exercise-induced changes in hydration status, we found that an acute bout of prolonged exercise resulted in tubular kidney injury, as evidenced by significant increase in uNGAL. In contrast to our hypothesis, we found no differences in renal responses between an acute bout *versus* repetitive bouts of exercise on three consecutive days, which indicates that there is no cumulative effect of kidney injury markers in response to repetitive exercise.

We found an increased uNGAL concentration after performance of single and repetitive bouts of exercise, which is indicative for acute kidney injury, and more specifically tubular injury[12, 13]. In a normal situation, NGAL is produced continuously at low levels by neutrophils of different tissues (*i.e.* colon-, trachea- and kidney epithelium), including the distal tubules[30, 31]. The circulating NGAL is filtered by the glomerulus and reabsorbed by the proximal tubule, resulting in low

urine concentrations[30, 32]. In case of acute kidney injury, the proximal tubular uptake of NGAL is impaired and the NGAL expression and release is upregulated in the distal tubule[12, 30]; both will increase the urinary excretion of NGAL[30], while the upregulated secretion of NGAL in the distal tubule is the primary source[32]. A cross-sectional study demonstrated that subjects with acute tubular necrosis had a more than 25-fold higher uNGAL concentration (normalized for urinary creatinine) compared to matched healthy controls (570 *versus* 23 ng/mL, respectively)[33]. We found a relatively small increase in uncorrected uNGAL (2.3-fold higher, 9.2 ng/mL at baseline to 20.7 ng/mL at day 1). Moreover, both baseline and post-exercise uNGAL concentrations were below the previously determined normal (23 ng/mL) and cut-off value (104 ng/mL) for acute kidney injury, in which acute kidney injury is defined as a 2-fold increase in serum creatinine or a 50% decrease in GFR[34, 35].

In a previous study the effect of marathon running[15] on uncorrected uNGAL concentrations was examined. They found a 5.7-fold increase in uNGAL concentration post-exercise compared to baseline (47.0 ng/mL). Our uncorrected values showed a 2.3 and 1.5-fold increase after day 1 and day 3, respectively. The lower average exercise intensity (71% *versus* 80%) and lower fluid balance disturbances (-0.9% *versus* -1.7%) in our study may partly explain the smaller increase in uNGAL[6, 18] compared to the observations in marathon runners. After correction for urine cystatin C concentration the uNGAL concentration showed a 1.2-fold increase, which suggests an even lower level of kidney injury. Therefore, our results suggest that an acute bout of exercise results in minor tubular kidney injury.

In contrast to uNGAL, uKIM1 levels did not differ between baseline and both exercise days after correction for cystatin C and creatinine. KIM1 is a type-1 transmembrane protein, which is absent in normal conditions, but elevated in the proximal tubule apical membrane cells after injury[25, 36]. In a previous study in marathon runners, post-exercise uKIM1 concentration was higher compared to baseline (3.5±1.6 ng/mL *versus* 2.6±1.6, respectively)[15]. Although kidney injury may have occurred, the reported increase may not be realistic as these data was not corrected for changes in hydration status. Our data underline the importance of this correction, as we found an increased uncorrected uKIM1 concentration after exercise day 1 as well, but the increase disappeared after cystatin C and creatinine corrections were applied. Moreover, with a similar correction for hydration status in the marathon runners, the exercise-induced increase in uKIM1 probably disappears. As in our study we found that creatinine and cystatin C corrected uKIM1 levels were not affected by a single or repeated bouts of moderate intensity endurance exercise, we conclude that exercise does not induce temporary injury to the proximal tubules.

The discrepancy in uNGAL and uKIM1 responses to exercise might be explained by a difference in etiology of release of both markers. KIM1 is undetectable in urine of normal kidneys[37] and the expression is increased in damaged proximal tubular cells and tubular inflammation[25],

whereas increased NGAL levels are associated with reduced proximal tubular uptake and injury of both the proximal and distal tubules[30]. The discrepancy between changes in concentrations of uKIM1 and uNGAL may indicate that, due to energetic stress, exercise-induced kidney injury primarily affects the distal tubule, leading to elevated secretion of NGAL[32]. In animal studies it was demonstrated that acute exercise, which consists of running on a treadmill at 1 km/h until exhaustion (mean time to exhaustion = 90 min), resulted in a significant increase in apoptotic cells in the kidney 2, 6 and 96 hours after cessation of exercise[8, 38]. Moreover, the apoptosis was only present in the distal tubulus and collecting duct of all exercising rats, whereas the presence of apoptosis was not demonstrated in proximal tubular cells[8, 38]. The authors suggested that the increased distal tubular apoptosis might be explained by transient renal ischemia as well as an increased expression of angiotensin II receptor type 1 and type 2 in distal tubular cells[38]. Moreover, after ischemic injury in the kidneys, apoptotic cells were prominent in distal tubulus[39]. The increased exercise-induced apoptosis of distal tubular cells, might explain the different response of uKIM1 and uNGAL in our study.

In contrast to our hypothesis, the uNGAL and uKIM1 concentration did not further increase after the first day of prolonged walking exercise. This may possibly be explained by the relatively short plasma half-life time of both NGAL (~15 min) [40] and KIM1 (~6 hours)[41], which would cause uNGAL and uKIM1 concentrations to drop back to baseline before onset of exercise on consecutive days. Furthermore, recent studies assessing myocardial dysfunction, using serum B-type Natriuretic Peptide[42] or serum troponin[43] (half-life time ~90-120 min), after four consecutive days of prolonged walking exercise did not find a difference in post-exercise values across the four days. Therefore, no accumulative effect of repetitive exercise was found with respect to myocardial dysfunction, which is in line with our results in which we demonstrate a comparable response of kidney injury to repetitive prolonged walking exercise on consecutive days.

We are the first to apply urinary cystatin C to correct for changes in hydration status. However, urinary cystatin C levels might be slightly elevated by exercise-induced proximal tubular stress[44], which questions the role as correction factor. However, the baseline and post-exercise urinary cystatin C levels found in our study are very low and fit well within the normal range (0.03 – 0.3 mg/L) described for non-exercising healthy subjects[44, 45]. These data underscore our indication above that the proximal tubular function is not affected in our two exercise bouts. Although the urinary creatinine concentration is commonly used in literature to correct for changes in hydration status, its application in exercise settings is less suitable due to the physiological muscle breakdown and related creatinine release during exercise[46]. Therefore, under non-steady state conditions, such as exercise, the urinary creatinine concentration changes over time, which probably affects the normalized level of both biomarkers[47]. The exercise-induced increase in urinary creatinine concentration after day 1 might, therefore, be caused by both dehydration and muscle breakdown. Furthermore, as a result of the high baseline and post-

exercise urine osmolality and the less controlled exercise conditions with different fluid and salt intake along subjects, the urine osmolality is our study design less suitable to normalize for changes in hydration status.

Clinical implications. A small exercise-induced increase in both corrected and uncorrected uNGAL was found after both day 1 and day 3, whereas corrected post-exercise uKIM1 levels did not differ from baseline. The uncorrected uNGAL levels, however, were below described cutoff values for acute kidney injury[34]. Our data, therefore, suggest that a single bout as well as repetitive bouts of exercise in subjects who are not diagnosed with kidney disease do lead to some kidney injury, but not to acute kidney injury. Furthermore, exercise-induced inflammation, which is represented by increased plasma IL-6 and TNF-α levels after exercise on day 1 and day 3, may also contribute to elevated plasma NGAL and consequently uNGAL concentrations[48]. However, we did not find a correlation between uNGAL concentration and the inflammatory markers IL-6 and TNF-α, which suggests that the increase in uNGAL is not associated with exercise-induced changes in inflammatory status. Furthermore, based on current study it is hard to establish whether the changes in uNGAL are the result of renal responses to the stress of exercise or the result of 'temporary' kidney injury or a combination of both.

The strength of the current study is the inclusion of a large group of subjects that performed prolonged exercise on three consecutive days, in which the urinary outcome parameters were corrected for dehydration-related changes in urine concentration. However, there are some limitations that should be taken into account. It is previously suggested that urine normalization to urinary flow rates is the best option to correct urinary outcomes for changes in hydration status. However, obtaining the urinary flow rate in field based settings is very hard and inconvenient to the subjects. Second, spot urines were used for all laboratory analyses. Although spot urines are well correlated with 24 hour urine samples and have the potential to operate as a surrogate for the preferred 24 hour urine collection, the use of spot urines is less accurate compared to a 24 hours urine collection[49]. However, urine collection during these walking marches is highly inconvenient to the subjects. Third, the average eGFR at baseline was 89.3±11.6 mL/min/1.73m^2. This relatively low eGFR might be explained by the previously described decrease in eGFR with aging[50].

In conclusion, an acute bout of prolonged moderate intensity exercise does not impact on uKIM1 levels corrected for hydration status in a middle-aged population, but does increase uNGAL, suggesting distal tubular injury. Moreover, no differences in renal responses are present between an acute bout *versus* repetitive bouts of exercise on three consecutive days, which indicates that there is no cumulative effect of kidney injury markers in response to repetitive exercise.

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Chapter 11

Impact of Acute versus Prolonged Exercise and Dehydration on Kidney Function and Injury

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Physiological reports

Impact of acute versus prolonged exercise on kidney function and injury markers

34 Male participants (23±3 years) completed a submaximal exercise protocol until 3% dehydration

Exercise intensity

80%

Part I:
Acute exercise

30 min

20°C

Part II:
Prolonged exercise

3% dehydration
or >120 min

25°C

Baseline

30 min

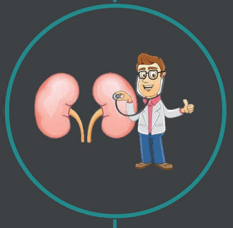
Acute exercise

110 min

Prolonged exercise

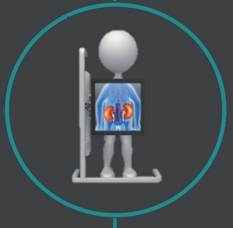
Dehydration - Body mass loss (%)

\- | 0.6±0.3% | 2.9±0.7%



Kidney Function - Glomerular filtration rate (mL/min)

118±11 | 116±12 | Not different | 103±16



Kidney injury - uNGAL (pg/mOsm)

2.8 (0.7-11.6) | 6.1 (1.5-16.7) | 16.5 (3.8-31.3)

Kidney injury - uKIM1 (pg/mOsm)

1.7 (0.3-3.4) | 2.6 (1.5-7.5) | Not different | 5.6 (2.1-11.6) | Not different

Acute exercise barely impact the kidneys, while prolonged exercise declines function and induces injury

ABSTRACT

Exercise and dehydration may be associated with a compromised kidney function and potential signs of kidney injury. However, the kidney responses to exercise of different durations and dehydration levels are not yet known. Therefore, we aimed to compare the effects of acute *versus* prolonged exercise and dehydration on estimated glomerular filtration rate (eGFR) and kidney injury biomarkers in healthy male adults. A total of 35 subjects (23±3 years) were included and invited for two study visits. Visit 1 consisted of a maximal cycling test. On Visit 2, subjects performed a submaximal exercise test at 80% of maximal heart rate until 3% dehydration. Blood and urine samples were taken at baseline, after 30 minutes of exercise (acute effects; low level of dehydration) and after 150 min of exercise or when 3% dehydration was achieved (prolonged effects, high level of dehydration). Urinary outcome parameters were corrected for urinary cystatin C, creatinine and osmolality. Subjects dehydrated on average 0.6±0.3% and 2.9±0.7% after acute and prolonged exercise, respectively ($p<0.001$). The $eGFR_{cystatin\ C}$ did not differ between baseline and acute exercise (118±11 *versus* 116±12mL/min/1.73m^2, $p=0.12$), whereas $eGFR_{cystatin\ C}$ was significantly lower after prolonged exercise (103±16mL/min/1.73m^2, $p<0.001$). We found no difference in osmolality corrected uKIM1 concentrations after acute and prolonged exercise ($p>0.05$), and elevated osmolality corrected uNGAL concentrations after acute and prolonged exercise (all p-values<0.05). In conclusion, acute exercise did barely impact on $eGFR_{cystatin\ C}$ and kidney injury biomarkers, whereas prolonged exercise is associated with a decline in $eGFR_{cystatin\ C}$ and increased biomarkers for kidney injury.

INTRODUCTION

Strenuous exercise increases the perfusion of active muscles, while the perfusion of body organs such as the kidneys may decrease up to 25% of resting levels[1, 2]. Furthermore, exercise increases the metabolic heat production, resulting in an elevated sweat rate and concomitant dehydration leading to a lower extracellular volume[3]. It has been hypothesized that the decreased renal blood flow and lower circulatory blood volume may attenuate kidney function and induce ischemic kidney stress or even 'temporary' kidney injury[4, 5].

Prolonged exercise accompanied with dehydration stimulates the secretion of arginine vasopressin (AVP)[6, 7] and activates the renin-angiotensin-aldosteron system (RAAS)[8], both stimulate the renal reabsorption of water and sodium chloride. The increased energy-demanding renal sodium uptake and the reduced renal perfusion with excessive dehydration may induce ischemic kidney injury[9]. Increased urinary levels of kidney damage biomarkers (kidney injury molecule 1 (KIM1) and neutrophil gelatinase-associated lipocalin (NGAL)) were found after completing a (ultra)marathon[10, 11] and after prolonged walking exercise[12]. However, the interpretation of these studies is difficult, as urinary KIM1 and NGAL concentrations were not corrected for elevated urine density as an effect of dehydration. Some studies corrected for changes in urinary density using urinary creatinine concentration[11, 13], which may be problematic because of exercise-induced muscle breakdown[13]. As a result, it is hard to distinguish whether observed changes are the effect of exercise or due to an increased urine concentration because of dehydration. In addition, previous studies primarily focused on the effects of prolonged exercise on kidney function and injury, but the acute effects of a short bout of exercise, with less dehydration, are unknown.

Therefore, the aim of this study was to assess and compare the effects of acute *versus* prolonged exercise on eGFR and kidney injury biomarkers in healthy male adults in well-controlled laboratory circumstances. To account for exercise-induced changes in urinary concentration, uNGAL and uKIM1 will be corrected for cystatin C, creatinine and osmolality. We hypothesized that acute exercise will not impact on eGFR and kidney injury, whereas prolonged exercise will result in a decreased eGFR and the presence of biomarkers for kidney injury. Furthermore, we hypothesize that higher levels of dehydration will augment the effects on eGFR and kidney injury biomarkers.

METHODS

Subjects

A total of 35 male subjects (23±3 years, 22.3±3.6 kg/m^2) between 18-30 years were included in this study. Subjects with a history of kidney disease or a baseline eGFR <90 mL/min were excluded for participation. The study was approved by the Medical Ethical Committee of the Radboud university medical center (CMO: 2015-1649), and all subjects gave written informed consent prior to participation. Furthermore, the study was conducted under the provisions of the Declaration of Helsinki.

Study design

All subjects were invited for two study visits, separated by at least 5 days, to prevent interference of the two visits. After given informed consent, subjects were medically screened to control for exclusion criteria and a venous blood sample was taken. Thereafter, a maximal exercise test was performed to determine subject's physical fitness level (VO_2 max) and maximal heart rate (HR max). The second study visit consisted of a submaximal exercise test on a cycle ergometer for 150 minutes or until 3% dehydration. At baseline, after 30 minutes of exercise (effects of acute exercise) and directly after exercise (effects of prolonged exercise), a blood and urine sample were taken and dry-toweled nude body mass was measured. Subjects were instructed to refrain from alcohol and caffeine consumption and heavy physical exercise 48 hours prior to the experiment. Furthermore, subjects were instructed to register all fluid intake 24 hours prior to the second study visit. To ensure that subjects were well hydrated before the exercise test, subjects were asked to drink 0.5 liter of water 2 hours prior to the test[14].

Study protocol

Study visit 1 – Medical screening + maximal exercise test. The medical screening consisted of a medical history check, a 12-lead ECG and physical examination. Subsequently, a venous blood sample was taken to determine the serum creatinine concentration, which was used to assess kidney function in rest by calculating the $eGFR_{creatinine}$. Furthermore, subjects were asked to complete the Short QUestionnaire to ASsess Health enhancing physical activity (SQUASH)[15]. This questionnaire takes into account sport activities as well as other daily activities such as work, housekeeping and leisure time activities. Subjects completed the questionnaire concerning an average week of the past 6 months. Furthermore, subjects completed a stepwise incremental cycling exercise protocol, in which the workload increased with 25 Watt per minute until volitional exhaustion, to determine subject's physical fitness (VO_2 max), HR max and maximal workload. Directly after volitional exhaustion we determined the rate of perceived exertion using a 10 point category Borg scale[16] and the blood lactate level using a fingertip capillary measurement (Lactate Pro, ARKRAY Europe, Amstelveen, The Netherlands).

Study visit 2 – Submaximal exercise test. During the second study visit, subjects performed a submaximal exercise test on a cycle ergometer at an exercise intensity of 80% of the HR max. At baseline the nude body mass, equipped body mass (body mass including sportswear and HR monitor), blood pressure and resting HR were measured. Furthermore, a venous blood sample was taken and subjects were asked to provide a urine sample and subsequently empty their bladder. Thereafter, subjects started the submaximal exercise test, which consisted of two parts. In the first part, subjects exercised at 80% of HR max for 30 minutes at a frequency of 60 to 80 repetitions per minute in an ambient temperature of 20°C. In the first 5 minutes of the exercise test, the workload was increased until the subjects reached 80% of their maximal heart rate. Thereafter, the workload was adjusted to maintain a constant exercise intensity of 80%. After 30 minutes, the baseline measurements were repeated and blood and urine samples were taken to assess the acute effects of exercise. Subsequently, subjects got dressed with a thermo suit (Craft active Basic, Craft, Beverly, Massachusetts, USA) and started the second part of the exercise protocol, in which the ambient temperature was elevated to 25°C. Subjects started cycling at 80% of HR max and continued exercise for another 120 minutes or until 3% dehydration (defined as a body mass loss of 3%) was achieved. The rate of perceived exertion (RPE) was measured on a 6-20 category Borg scale. Furthermore, the equipped body mass, ambient temperature and relative humidity were measured every 15 minutes. Directly after completing the submaximal exercise test, the blood pressure was measured and blood and urine samples were taken again. Thereafter, the nude body mass was measured to determine the relative body mass loss (dehydration).

Study parameters

Fluid balance. Nude body mass was measured at baseline, after 30 minutes and directly after finishing the submaximal exercise test. Subsequently, the exact level of dehydration was calculated as the relative change in nude body mass (in %). Furthermore, all subjects received written and individual oral instruction about the registration of their fluid intake in a diary. Subjects were allowed to drink ad libitum, as long as they registered the time (in blocks of 1 hour), amount (standardized sized cups, bottles, etc.) and type (water, sports drink, tea) of their individual fluid intake 24 hours preceding the submaximal exercise test.

Blood sample. A blood sample was taken at baseline, after 30 minutes and directly after completing the submaximal exercise test. Serum creatinine and cystatin C concentrations were determined to examine kidney function. In literature, the Chronic Kidney Disease Epidemiology Collaboration formula (CKD-EPI) based on creatinine is often used to determine kidney function[17]. However, serum creatinine levels increase during exercise due to exercise-induced muscle breakdown. In contrast, cystatin C is independent of muscle mass, age and gender, and serum levels are not influenced by exercise[18]. Therefore, we used the CKD-EPI formula based on creatinine[17] as well as the CKD-EPI formula based

on cystatin C[18] to calculate the eGFR at rest and after acute and prolonged exercise. Additionally, serum osmolality and plasma sodium concentration were measured as indices for dehydration. Plasma haematocrit and haemoglobin levels were measured and used to calculated the relative changes in plasma volume (in %) based on the Dill and Costil formula[19]. Furthermore, the plasma renin activity (PRA) and copeptin concentration were measured, to assess the hormonal responses to changes in the volume and osmolality balances, respectively. Arginine vasopressin (AVP) is relatively difficult to measure because of its short half-life and its interaction with blood platelets[20]. Copeptin and AVP are derived from the same pre-prohormone and thus synthesized and secreted in equal molar amounts. Copeptin, however, has a longer half-life and can be measured more easily than AVP[20]. Therefore, we measured copeptin as a surrogate marker for AVP secretion[21].

Urine sample. At baseline, after 30 minutes and directly after completing the submaximal exercise test, all subjects provided a urine sample to examine fluid balance and kidney responses. Urinary cystatin C concentration was measured using the nephelometric method (Behring Nephelometer II, Siemens Healthcare, Den Haag, The Netherlands). The urinary creatinine concentration was measured using an enzymatic assay (Cobas C6000, Roche Diagnostics, Indianapolis, USA). Urine osmolality was examined using an osmometer (Advanced Model 3320 Micro-Osmometer, Osmometer, Advanced Instruments, Norwood, USA).

In order to determine kidney injury in response to exercise, we measured urinary concentrations of KIM1 and NGAL (both monomeric and dimeric) in duplicate using the previously described sandwich ELISA assay (E-EL-H0186 and E-EL-H0096, Elabscience Biotechnology, Wuhan, China)[22, 23]. Furthermore, uKIM1 and uNGAL concentrations were corrected for urinary cystatin C, creatinine and osmolality. The corrected uKIM1 and uNGAL data were calculated by dividing the individual uncorrected data by the corresponding cystatin C levels, creatinine levels and the urine osmolality. Additionally, urinary albumin (uAlbumin) and glucose (uGlucose) concentrations were measured, and corrected as well, to examine the effects of acute exercise and dehydration on acute kidney injury.

Exercise intensity. Subjects HR was measured continuously every 15 seconds throughout the test using a two-channel chest band system (Polar RS400, Polar, Oy, Kempele, Finland). Subsequently, the exercise intensity was calculated by expressing the HR as a percentage of HR max.

Statistical analysis

The statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS version 20, Armonk, NY, USA), in which the level of significance was set at $p<0.05$. Data was checked for normality using the Shapiro-Wilk test. In case of a non-Gaussian distribution, the statistical analysis was performed using the non-parametric equivalents. Normally

distributed data were presented as mean ± standard deviation (SD), whilst non-Gaussian distributed date were presented as median (interquartile range). A repeated measures ANOVA was used to assess differences in fluid balance, kidney function and kidney injury over time, in which a post-hoc Bonferroni correction was used to determine individual differences between baseline, acute exercise and prolonged exercise. Non-Gaussian distributed data were tested using a Friedman test, followed by a Wilcoxon signed rank test. Furthermore, we have calculated the absolute change in uKIM1 and uNGAL compared to baseline for both acute (Δ acute exercise) and prolonged exercise (Δ prolonged exercise). Subsequently, we used a paired t-test or Wilcoxon signed rank test to assess whether there is a difference between Δ acute exercise and Δ prolonged exercise. A Pearson or Spearman (for non-Gaussian distributed data) correlation coefficient was used to assess the relation between kidney function and injury and level of dehydration.

RESULTS

Subjects & exercise characteristics. An overview of subject characteristics is shown in Table 1. Because of a vasovagal syncope in response to the venous blood sample, one subject did not perform the submaximal exercise test. Average fluid intake 24 hours prior to the test was 2,956±947 mL. The average exercise duration during the submaximal exercise was 107±16 minutes, in which subjects cycled at an exercise intensity of 79.3±1.4% of HRmax. The average workload in the first part was significantly higher compared to the second part of the submaximal exercise test (152±30 W *versus* 116±36 W, p<0.001), with an average exercise intensity of 79.8±3.5% and 81.1±1.0% for the first and second part, respectively (p=0.016). Furthermore, the ambient temperature and relative humidity were, respectively, 20±1°C and 61±14% for the first part and 25±1°C and 59±12% for the second part of the submaximal exercise test.

Fluid balance. Fluid balance data are shown in Table 2. Relative body mass loss after acute and prolonged exercise was 0.6±0.3% and 2.9±0.7%, respectively (p<0.001), while the decrease in plasma volume was also higher after prolonged exercise (p<0.001). An exercise-induced increase in urine osmolality was observed after both acute and prolonged exercise (both p-values <0.001). No differences in serum sodium concentration and serum osmolality were found after acute exercise (p>0.05), whereas sodium concentration and serum osmolality increased after prolonged exercise (p<0.001). Plasma copeptin concentration after acute exercise did not increase (p=0.07), whereas a significant increase was found after prolonged exercise (p<0.001). Furthermore, baseline PRA levels increased after both acute and prolonged exercise (both p-values <0.001), with higher PRA levels after prolonged exercise compared to acute exercise (p<0.001).

Table 1. Subject characteristics and results of maximal exercise test (n=34)

Parameter	Total group (n=34)
Subject characteristics	
Age (years)	22.8±2.9
Length (m)	1.83±0.06
Body mass (kg)	74.6±10.5
BMI (kg/m^2)	22.5±3.6
Systolic blood pressure (mmHg)	128±10
Diastolic blood pressure (mmHg)	69±9
Resting heart rate (bpm)	66±14
Serum Creatinine (µmol/L)	84.3±8.9
$eGFR_{creatinine}$ (mL/min/1.73 m^2)	110.8±11.0
Activity score (au)	8,579±4,127
Maximal exercise test	
VO_2 max (mL/min/kg)	56.6±10.6
HR max (bpm)	194±9
Maximal workload (W)	338±55
Blood lactate level (mmol/L)	12.9±1.8
RER (ratio: VCO_2/VO_2)	1.18±0.07
Rate of perceived exertion (au)	8.2±1.2

Subject characteristics for the total group. Data were presented as mean±SD. MET= Metabolic equivalent of task, eGFR= estimated glomerular filtration ratio, bpm= beats per minute, au= arbitrary unit.

Table 2. Fluid balance responses

Parameter	Baseline	Acute exercise	Prolonged exercsie	p-value
Relative body mass loss (%)	-	0.6±0.3[a]	2.9±0.7[a,b]	**p<0.001**
Plasma hemoglobin (mmol/L)	9.2±0.7	9.6±0.6[a]	9.7±0.6[a,b]	**p<0.001**
Plasma hematocrit (L/L)	0.47±0.03	0.48±0.03[a]	0.49±0.03[a]	**p<0.001**
Plasma volume loss (%)	-	3.5±2.1	4.8±2.2[b]	**p<0.001**
Urine Osmolality (mOsm/kg)	364 (201–624)	585 (360-735)[a]	837 (728-961)[a,b]	**p<0.001**
Serum Osmolality (mOsm/kg)	293±7	294±6	300±6[a,b]	**p<0.001**
Serum Sodium (mmol/L)	142.2±2.3	141.9±2.7	144.0±2.6[a,b]	**p<0.001**
Plasma copeptin (pmol/L)	4.6 (3.3-7.4)	6.6 (4.2-13.5)	35.9 (25.7-47.6)[a,b]	**p<0.001**
Plasma renin activity (pmol/L)	1.6 (1.1-2.6)	4.5 (3.2-5.7)[a]	13.4 (9.9-19.0)[a,b]	**p<0.001**

Data were presented as mean±SD or median (interquartile range). [a] significantly different from baseline, [b] different from acute exercise

Kidney function. An exercise-induced increase in serum creatinine concentration was found after both acute (p=0.011) and prolonged exercise (p<0.001), with higher levels after prolonged compared to acute exercise (p<0.001). Serum cystatin C levels were comparable between baseline and acute exercise (p=0.36), whereas an increased cystatin C concentration was found after prolonged exercise (p<0.001). Baseline eGFRcreatinine was 114 (102 – 123) mL/min/1.73 m2 and decreased to 109 (95 – 122) mL/min/1.73 m2 after acute exercise (p=0.009), with a further decrease to 98 (82 – 105) mL/min/1.73 m2 after prolonged exercise (p<0.001). In contrast, baseline eGFRcystatin C did not change after acute exercise (118±11 mL/min/1.73 m2 versus 116±12 mL/min/1.73 m2, p=0.12), whereas a significant decrease was found after prolonged exercise (103±16 mL/min/1.73 m2, p<0.001, Figure 1). Furthermore, no correlation was found between eGFRcystatin C and level of dehydration after acute (R2= 0.03, p=0.30) and prolonged exercise (R^2= 0.01, p=0.54).

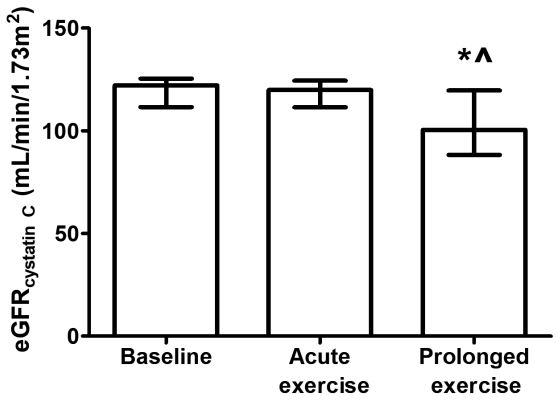


Figure 1. The estimated glomerular filtration ratio calculated with the cystatin C formula at baseline and after acute and prolonged exercise. Data were presented as median with interquartile range. * Represents a difference between acute and prolonged exercise.

Urinary kidney injury markers. Due to technical difficulties the data analysis of one of the badges of uKIM1 failed and uKIM1 cannot be determined in n=9 subjects. Furthermore, n=3 subjects were not able to provide a urine sample after 30 minutes of exercise. A significant increase in urinary creatinine and cystatin C concentration was found after acute and prolonged exercise (all p-values <0.05), with higher levels after prolonged compared to acute exercise (Table 3).

Table 3. Urinary outcome parameters

Parameter	Baseline	Acute exercise	Prolonged exercsie	p-value
uCystatin C (mg/L)	0.01 (0.01-0.04)	0.03 (0.01-0.08)[a]	0.15 (0.09-0.26)[a,b]	**<0.001**
uCreatinine (mmol/L)	5.0 (3.3-14.5)	9.2 (5.5-19.1)[a]	26.3 (20.5-37.8)[a,b]	**<0.001**
uAlbumin (mg/mL)	3.9 (2.1-7.1)	10.0 (3.8-23.2)[a]	32.5 (16.4-50.1)[a,b]	**<0.001**
uAlbumin (mg/µg Cystatin C)	308 (130-425)	313 (150-821)	161 (119-553)	**0.14**
uAlbumin (mg/mg Creatinine)	6.0 (3.9-11.0)	7.3 (5.3-14.4)	7.9 (6.0-12.8)[a]	**<0.001**
uAlbumin (µg/mOsm)	11.7 (7.6-21.0)	17.5 (9.6-36.4)[a]	35.1 (20.9-63.4)[a,b]	**<0.001**
uGlucose (mmol/L)	0.11 (0.10-0.28)	0.17 (0.11-0.33)[a]	0.50 (0.33-0.79)[a,b]	**<0.001**
uGlucose (mmol/mg Cystatin C)	5.7 (2.7-12.0)	5.5 (3.5-17.0)	3.1 (2.1-4.0)[a,b]	**<0.001**
uGlucose (mmol/g Creatinine)	0.19 (0.15-0.24)	0.18 (0.17-0.22)	0.16 (0.13-0.18)[a,b]	**0.023**
uGlucose (µmol/mOsm)	3.4 (2.4-4.8)	3.4 (2.7-5.1)	5.4 (4.4-8.9)[a,b]	**0.026**

Data were presented as mean±SD or median (interquartile range).[a] Significantly different from baseline, [b] different from acute exercise

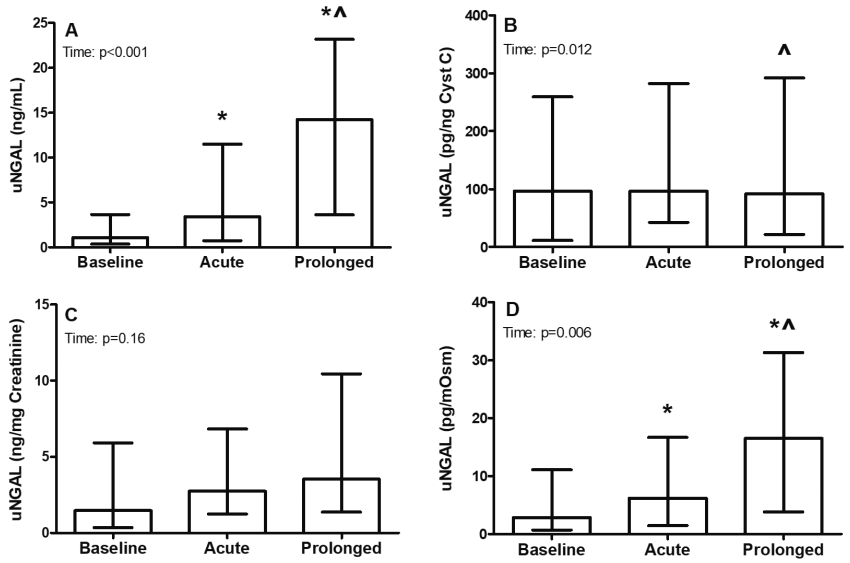


Figure 2. Urinary NGAL concentration uncorrected **(A)**, as well as after correction for cystatin C **(B)**, creatinine **(C)** and osmolality **(D)**, at baseline and after acute and prolonged exercise (n=31). A Friedman test was used to examine differences in uNGAL over time, whereas a Wilcoxon signed-rank test was used to assess differences acute and prolonged exercise. Data were presented as median (interquartile range) for uncorrected and creatinine and osmolality corrected. * Represents a significant difference from baseline, and ^ represents a difference from acute exercise.

Table 4. Changes in uKIM1 and uNGAL compared to baseline

Parameter	Δ Acute exercise	Δ Prolonged exercise	p-value
uKIM1 (ng/mL)	0.7 (-0.2 - 2.1)	3.0 (0.8 - 7.6)	**0.003**
uKIM1 (pg/ng Cystatin C)	4.2±72.0	-20.1±59.2	**0.01**
uKIM1 (ng/mg Creatinine)	0.1 (-0.9 - 1.0)	0.1 (-0.9 - 1.5)	0.69
uKIM1 (pg/mOsm)	0.8 (-1.1 - 2.4)	2.7 (-0.1 - 8.2)	**0.022**
uNGAL (ng/mL)	1.0 (0.0 - 8.0)	11.1 (0.7 - 22.6)	**0.001**
uNGAL (pg/ng Cystatin C)	30.1 (-49.5 - 84.3)	-15.1 (-86.5 - 55.8)	**0.044**
uNGAL (ng/mg Creatinine)	1.8±4.6	2.9±8.8	0.58
uNGAL (pg/mOsm)	2.7 (-0.5 - 8.01)	8.0 (0.2 - 28.6)	**0.022**

Data were presented as mean±SD or median (interquartile range).

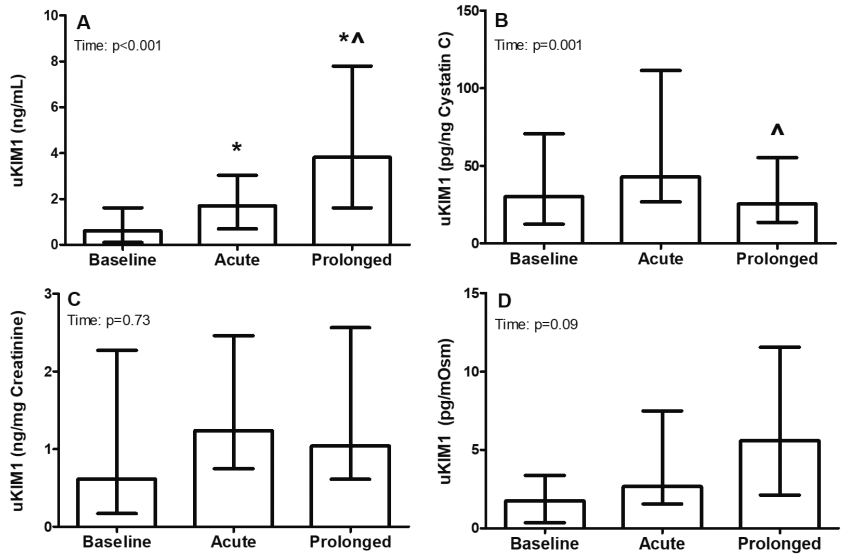


Figure 3. Urinary KIM1 concentration uncorrected **(A)**, as well as after correction for cystatin C **(B)**, creatinine **(C)** and osmolality **(D)**, at baseline and after acute and prolonged exercise (n=22). A Friedman test was used to examine differences in uKIM1 over time, whereas a Wilcoxon signed-rank test was used to assess differences acute and prolonged exercise. Data were presented as median (interquartile range). * Represents a significant difference from baseline, and ^ represents a difference from acute exercise.

Urinary NGAL. The uncorrected uNGAL concentration increased after both acute (p=0.001) and prolonged exercise (p<0.001), with higher levels after prolonged exercise (p=0.001, Figure 2).The cystatin C corrected uNGAL concentration did not differ after acute or prolonged exercise (both p-values >0.05), with lower levels after prolonged compared to acute exercise (p=0.044). No difference in creatinine corrected uNGAL (p=0.16) concentrations were found across measurements. The osmolality corrected uNGAL concentration also increased after both acute (p=0.018) and prolonged exercise (p<0.001), with higher levels after prolonged exercise (p=0.022). The Δ prolonged exercise was significantly higher compared to Δ acute exercise for uncorrected uNGAL and cystatin C and osmolality corrected uNGAL (all p-values <0.05), whereas no difference between acute and prolonged exercise in change in creatinine corrected uNGAL was found (p=0.58, Table 4). After both acute and prolonged exercise, no correlation was found between the uncorrected and corrected uNGALconcentrations and dehydration level and absolute body mass loss (all p-values>0.05).

Urinary KIM1. The uncorrected uKIM1 concentration increased after both acute (p=0.021) and prolonged exercise (p<0.001), with higher levels after prolonged exercise (p=0.003, Figure 3). Cystatin C corrected uKIM1 was comparable after acute exercise (p=0.52) and prolonged exercise (p=0.062), with lower levels after prolonged compared to acute exercise (p=0.003). Creatinine and osmolality corrected uKIM1 levels did not differ across measurements (p=0.73 and p=0.09, respectively). The Δ prolonged exercise was significantly higher compared to Δ acute exercise for uncorrected uKIM1 and cystatin C and osmolality corrected uKIM1 (all p-values <0.05), whereas no difference between acute and prolonged exercise in absolute change in creatinine corrected uKIM1 was found (p=0.69, Table 4). A weak, but statistically significant, negative correlation was found between the uncorrected uKIM1 concentration and level of dehydration after acute exercise (R2= -0.46, p=0.029) and a positive correlation was found between creatinine corrected uKIM1 and level of dehydration after prolonged exercise (R^2= 0.45, p=0.022).

Urinary Albumin. The uncorrected uAlbumin concentration significantly increased after both acute (p<0.001) and prolonged exercise (p<0.001), with higher levels after prolonged exercise compared to acute exercise (p<0.001). Cystatin C corrected uAlbumin levels did not differ across measurements (p=0.14). Creatinine corrected uAlbumin levels did not differ after acute exercise (p=0.13), whereas increased levels were found after prolonged exercise (p=0.005). The osmolality corrected uAlbumin concentration was increased after both acute (p=0.028) and prolonged exercise (p<0.001), with higher levels after prolonged exercise (p=0.027).

Urinary Glucose. The uncorrected uGlucose concentration increased after both acute (p=0.024) and prolonged exercise (p<0.001), with higher levels after prolonged exercise (p<0.001). Furthermore, the cystatin C, creatinine and osmolality corrected uGlucose concentration

did not differ from baseline after acute exercise (all p-values >0.05), whereas the cystatin C, creatinine and osmolality corrected uGlucose levels were higher compared to baseline after prolonged exercise (all p-values <0.05).

DISCUSSION

This is the first study that makes a direct comparison of the effects of acute *versus* prolonged exercise on eGFR and kidney injury biomarkers in healthy male adults. Furthermore, we are the first to use urinary cystatin C and osmolality to correct urinary biomarkers for changes in hydration status. We found that the $eGFR_{cystatin\ C}$ did not change after acute exercise, whereas it significantly decreased after prolonged exercise. Furthermore, the uncorrected uKIM1 concentration and uncorrected and osmolality corrected uNGAL concentrations were elevated after both acute and prolonged exercise, with higher levels after prolonged compared to acute exercise. Moreover, Δ prolonged exercise was significantly higher compared to Δ acute exercise for uncorrected, cystatin C corrected and osmolality corrected uKIM1 and uNGAL. These results suggest that acute exercise as well as prolonged exercise may be associated with kidney injury, in which lower levels of kidney injury biomarkers were found after acute compared to prolonged exercise.

The absence of a decrease in $eGFR_{cystatin\ C}$ after a short bout of exercise suggests that the kidneys are well able to maintain kidney function in response to exercise and small perturbations in fluid balance. Literature reveals that the filtration fraction increases during exercise in response to a drop in renal blood flow, in which the increase in filtration fraction is caused by an increased vasoconstriction of the efferent arteriole[2]. As a result, the secretion of nitric oxide and prostaglandin E2 by the macula densa is upregulated, which results in a dilation of both the afferent and efferent arteriole and restoration of GFR[2, 24, 25]. After prolonged exercise we did find a decrease in $eGFR_{cystatin\ C}$, which is in line with previous studies with long distance runners and cyclists that demonstrated similar decreases in $eGFR_{creatinine}$ post-exercise, which were restored 24 h post-exercise[26-28]. It has been suggested that the transient alterations in eGFR are associated with the post-exercise hydration status, but it may also be affected by exercise-induced inflammation or oxidative stress[26]. We did not find a correlation between the level of dehydration and $eGFR_{cystatin\ C}$ after both acute and prolonged exercise. The strain of dehydration, inflammation and oxidative stress is lower after acute compared to prolonged exercise, which might suggest that the kidneys are well able to preserve $eGFR_{cystatin\ C}$ after acute exercise, while $eGFR_{cystatin\ C}$ declines after prolonged exercise.

The measurement of urinary biomarkers is likely to be influenced by changes in hydration status. Dehydration may impact urine concentration, which subsequently may overestimate the concentration of injury markers. Therefore, it is necessary to correct urinary biomarkers

for changes in hydration status. We used urinary creatinine, cystatin C and osmolality to correct our findings. Previous studies demonstrated, however, that serum and urine concentrations of creatinine may increase as a consequence of exercise-induced muscle breakdown[13]. On the other hand, urinary cystatin C levels may slightly increase as a consequence of a decreased proximal reabsorption due to kidney stress[29]. We used urinary cystatin C and creatinine concentrations to correct for changes in hydration status, while these correction methods both have limitations, since it may lead to an underestimation of the true effect of exercise on kidney injury. Alternatively, urine osmolality may be a better option to correct the data for hydration status. The urine osmolality is the most accurate measurement of total solute concentration and it therefore provides the best measurement of the kidney's concentrating ability[30]. As a result, the urine osmolality has previously been established as a valid measure for hydration status[31], which might be used as a correction method. Therefore, we will discuss our results with respect to kidney injury based on the osmolality corrected data.

We found increased urine osmolality corrected uNGAL levels after both acute and prolonged exercise, while the urine osmolality corrected uKIM1 concentration tended to be higher after prolonged exercise. These results suggests an exercise-induced development of kidney injury as a consequence of exercise-induced kidney stress. This is further supported by increased osmolality corrected uAlbumin and uGlucose levels after acute and prolonged exercise, which suggests that the proximal reabsorption of both substances is deteriorated as a consequence of kidney stress[32, 33]. In resting conditions, the proximal tubules almost completely reabsorb the NGAL that is produced continuously at low levels by neutrophils of different tissues (i.e. colon-, trachea- and kidney epithelium)[34, 35]. Additionally, the distal tubules secrete low levels of NGAL as well, resulting in low urinary concentrations[35, 36]. In case of kidney stress, the proximal tubular uptake of NGAL is impaired and the NGAL expression and release are upregulated in the distal tubule[35, 37]. Both will increase the urinary excretion of NGAL, but the upregulated secretion of NGAL by the distal tubules is the primary source[34]. Therefore, the elevated osmolality corrected uNGAL levels after acute and prolonged exercise, as found in our study, suggest proximal tubular injury. Our findings are in line with previous studies that demonstrated increased uKIM1 and uNGAL levels after (ultra) marathon running[10, 11, 38]. However, the post-exercise uncorrected uNGAL level after prolonged exercise found in our study (16.5 ng/mL) was lower compared to previous studies with post-exercise values ranging from 37.6 - 47.0 ng/mL[10, 11, 38]. This might be explained by our relatively young population (23 years *versus* >38 years), and the shorter period of exercise in our study (137 min *versus* >240 min). Moreover, the uncorrected uNGAL levels in the present study were far below the cutoff value (104 ng/mL) that has been used to diagnose kidney injury in clinical settings[39]. Therefore, moderate intensity exercise in young individuals results in subclinical kidney injury.

Clinical Relevance. Our results demonstrate that healthy young male adults are well able to maintain $eGFR_{cystatin\ C}$ after acute exercise, whereas an average decline of 15.4 mL/min/1.73 m^2 (13.2%) and a largest decline of 43.2 mL/min/1.73 m^2 (35.3%) was found after prolonged exercise. Our results suggest that prolonged exercise with ~3% dehydration induces kidney stress, which result in kidney injury, as shown by increased osmolality corrected uKIM1 and uNGAL levels. Next to the detrimental effects of prolonged exercise and dehydration on kidney injury, previous studies also demonstrate that kidney injury might be influenced by heat stress, systemic inflammation and renal perfusion[40-42]. One might argue that the impact of heat stress, inflammation and renal perfusion is higher after prolonged compared to acute exercise. Therefore, it is hard to establish whether the increase in kidney injury biomarkers can be explained by prolonged exercise with ~3% dehydration solely, or by a combination of exercise duration, dehydration, heat stress, inflammatory state and renal perfusion. Future studies should therefore further elaborate on the relationship between exercise and kidney injury, in which more attention should be given to individual factors that influences kidney responses to exercise.

The strength of the current study is the well-controlled study design, in which subjects performed a continuous exercise bout at a constant workload with increasing levels of dehydration. However, there are some limitations that should be taken into account. First, by the absence of a measurement 24 hours post-exercise we were not able to determine whether the decline in kidney function and the induced kidney injury are temporary. However, previous studies demonstrated that the decline in kidney function and the increases in kidney injury markers already restored after 24 hours of recovery[5, 10]. Second, spot urines were used for all laboratory analyses. Although spot urines correlate well with 24 hours urine samples and have the potential to operate as a surrogate for the preferred 24 hours urine collection, the use of spot urines is less accurate compared to a 24 hours urine collection[43]. Moreover, the total urine volumes at baseline and after acute and prolonged exercise were not determined. Therefore, the urinary flow rate and urinary filtration over a period of time, which are potentially the best options to correct for changes in hydration status, cannot be calculated. Furthermore, within this study we did not measure core body temperature or inflammatory markers, while these factors can exacerbate kidney stress and elevate biomarkers for kidney injury.

In conclusion, our results suggest that, in a group of healthy young male subjects, acute exercise barely impact on $eGFR_{cystatin\ C}$ and biomarkers for kidney injury, whereas prolonged exercise is associated with a decline in $eGFR_{cystatin\ C}$ and a further increase in biomarkers for kidney injury. follow-up studies are warranted to determine whether prolonged exercise-induced acute kidney injury is primarily due to exercise duration, dehydration, heat stress and/or inflammation.

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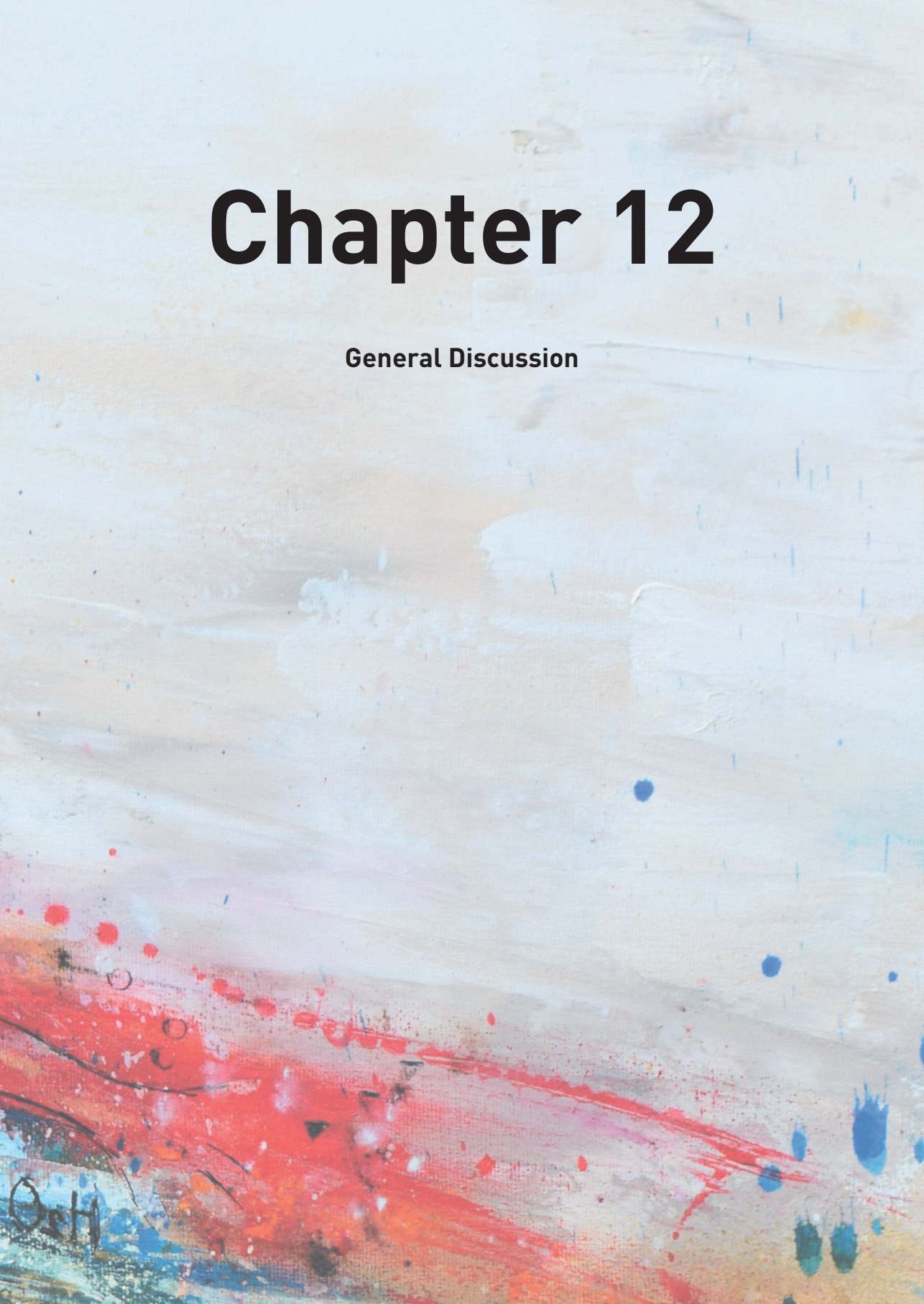
Chapter 12

General Discussion



The increased metabolic heat production and sweat rate associated with strenuous activities creates a major physiological challenge for the exercising athlete. Accordingly, exercise-induced hyperthermia and dehydration, particularly in a hot and humid environment, reduces exercise performance and increases the risk for heat-related illnesses. The general aim of this thesis was to examine thermoregulatory and fluid balance responses to exercise. Second, we aimed to determine the most beneficial cooling strategy to improve exercise performance in the heat. In this final chapter, our results are summarized and the consequences of our findings will be discussed.

Thermoregulation, Fluid balance and Exercise intensity

The core body temperature (Tc) increases during exercise[1-3], which can lead to the development of heat-related illnesses or death[4, 5]. The major (~80%) heat dissipating mechanism during exercise in the heat is the evaporation of sweat[6-8], which can result in disturbances in fluid balance and dehydration[6]. Dehydration may impact on the increase in Tc as well, in which an additional increase in Tc of ~0.15°C per 1% dehydration has been reported.[9, 10]. The metabolic heat production during exercise is mainly dependent on exercise intensity. Moreover, the heat production is directly proportional to the muscle mass that supports the movement, in which more muscles were involved to perform exercise at a higher intensity[1, 7, 11]. This is in line with the observed thermoregulatory responses in previous work of our department and results of this thesis (Figure 1). In *Chapter* 9, elderly (60±1 year) of the Four Days Marches demonstrated a finish Tc of 38.2±0.3°C after completing a 30-km walking exercise at an exercise intensity of 70±9% of maximal heart rate. In *Chapter 7* we found an average finish Tc of 39.1±0.5°C after completing a 5-km running time trial at an average intensity of ~95% of the maximal estimated heart rate (Figure 1). The exercise intensity is, therefore, strongly associated with the thermoregulatory burden of exercise, while a higher exercise intensity is also associated with more severe dehydration[6]. This association suggests that there is a continuous interaction between exercise intensity, thermoregulation and fluid balance, which may impact on exercise performance (Figure 2). The drop in performance with exertional hyperthermia and severe dehydration[12-15] can be the result of a downregulation of skeletal muscle activity and thereby exercise intensity[12, 16]. Based on the results of this thesis we suggest that cooling athletes prior to or during exercise can reduce the thermoregulatory strain. This enables athletes to maintain a high exercise intensity for a longer duration, which can improve their exercise performance. Therefore, cooling strategies directly impact on the interaction between thermoregulation, fluid balance and exercise intensity (Figure 2).

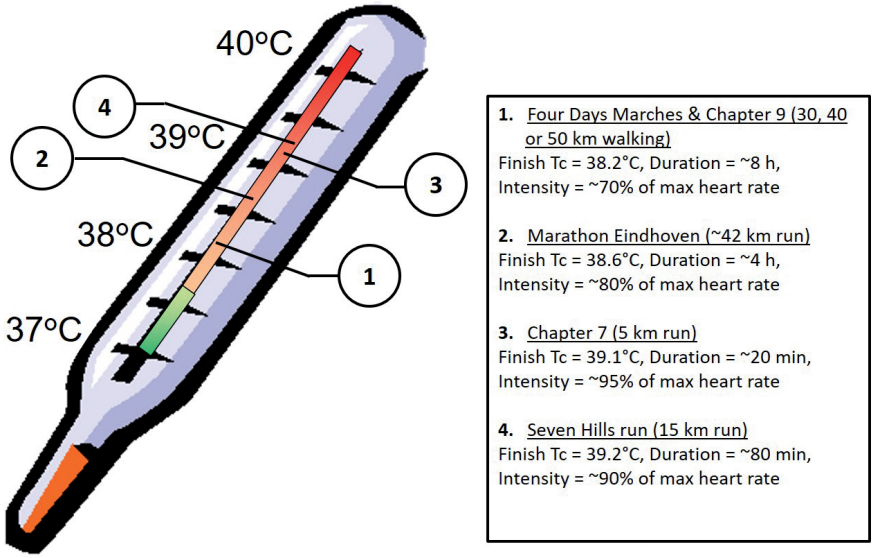


Figure 1. Overview of exercise-induced changes in Tc during different exercise events.

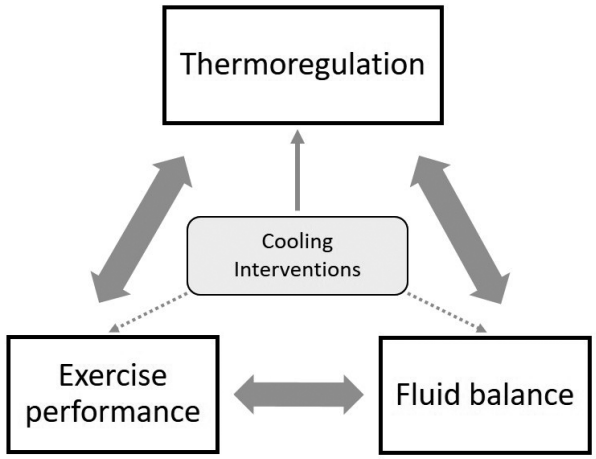


Figure 2. Schematic overview of the continuous interaction between thermoregulation, fluid balance and exercise performance. Cooling interventions directly impact on the thermoregulation (solid arrow) and have an indirect, via the thermoregulation, influence on the fluid balance and exercise performance (dotted arrow).

Individual thermoregulatory responses

In literature different subject characteristics have been associated with an increased risk to develop exertional hyperthermia. Advanced aging, low physical activity level, non-acclimatized status, female gender and a high body mass index, will lead to a lower heat loss capacity, resulting in a higher risk to develop hyperthermia[17, 18]. Additionally, individuals with a spinal cord injury have an attenuated sweating response and vasomotor control below the level of the lesion and are therefore more prone to develop exertional hyperthermia[19, 20]. Other factors that influences the increase in Tc relate to the ambient temperature, relative humidity, wind velocity and clothing[17, 21, 22] (Figure 3). However, for elite athletes in competitive settings the factors mentioned above are usually broadly similar, whereas the thermoregulatory burden of exercise, represented by an increase in Tc, is still different among those athletes. Previous literature revealed a large inter-individual variability in Tc elevation following exercise[23]. Therefore, it is hard to predict which athlete is at risk to develop exertional hyperthermia and will show associated performance decrements during exercise. As a result of the large inter-individual variability in Tc elevation following exercise and associated performance loss, athletes and coaches are increasingly interested in monitoring Tc as well as in interventions to reduce the thermal strain of exercise.

A continuous and real time measurement of Tc during exercise in field-based settings is therefore essential to prevent performance loss and the development of heat-related illnesses or even death. Ingestible telemetric temperature capsules have been introduced to examine gastrointestinal temperature accurately and relatively non-invasive(24, 25). In Chapter 3 and 4 we demonstrated that the recently developed myTemp temperature capsule has an excellent validity, reliability and response time to changes in water temperature in well-controlled ex-vivo circumstances. Moreover, this capsule system is relatively cheap, easy in use (plug and play) and does not have restrictions related to battery lifetime, disposability and expiry. The introduction of cheap and accurate ingestible temperature capsules could be a first step into the wide implementation of temperature capsules to measure changes in Tc for both scientific and non-scientific (*i.e.* elite athletes, large exercise events and clinical settings) purposes. The measured Tc can for example be displayed on a smart watch using smart technology, which enables athletes and coaches to receive real time information regarding their Tc. Based on this information, an athlete can adjust his/her pacing and/or cooling strategy, or the team doctor could intervene in case of a critical high Tc. Furthermore, since the increase in Tc is remarkably reproducible within athletes(26), the ingestible temperature capsule can also be used to repeatedly measure an athlete's Tc during exercise at different intensities, durations and/or ambient conditions, which can be used to create an individual thermoregulatory risk profile. Based on this information one can decide on an individual level whether cooling or other strategies to reduce thermal strain (*i.e.* pacing, clothing, acclimatization) could be beneficial to improve exercise performance. Furthermore, based on their individual temperature profile, athletes can experiment with different cooling strategies and cooling interventions to determine their best

individual strategy to improve exercise performance. For future perspective, the gastrointestinal temperature system might be incorporated into smart clothing, which should ideally have cooling capacity as well. Within this smart clothing the Tc can be measured continuously and the amount of cooling can be adjusted to the rise in Tc. This type of clothing can be used during exercise events to decrease the decline in performance and reduce the risk to develop heat-related illnesses.

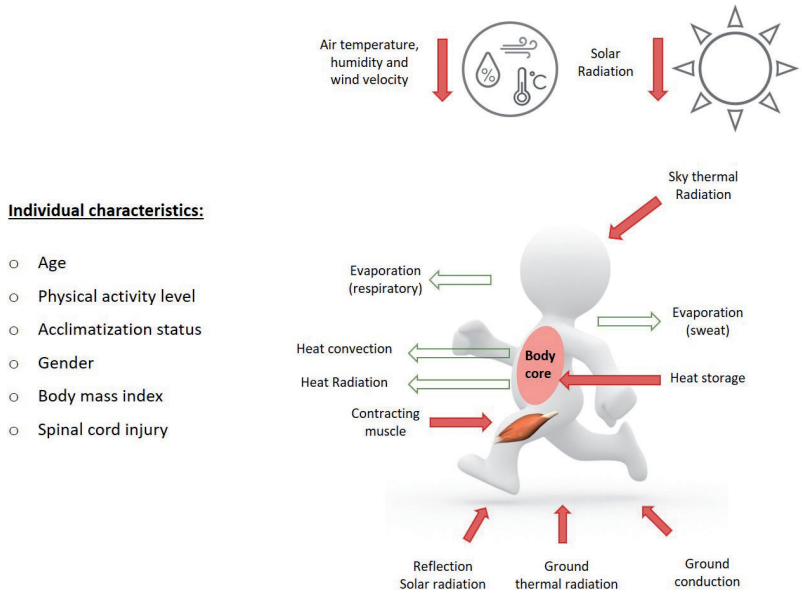


Figure 3. Overview of factors that influence the thermoregulation during exercise in the heat, in which the grey arrows represents heat storage and the white arrows represents heat loss.

Cooling interventions

The use of cooling techniques has shown to be effective to attenuate the increase in Tc and improve exercise performance[27, 28]. In this thesis we aimed to get a better insight into the potential benefits of cooling techniques, which was necessary to identify 'best practice' to improve thermal comfort during exercise and exercise performance. We found that pre-cooling and per-cooling are equally effective in improving exercise performance in the heat (ambient temperature >30°C). Furthermore, we identified mixed method cooling (use of multiple techniques) as the most effective pre-cooling strategy, in which cold water immersion is the most effective single pre-cooling strategy. In contrast, cold water or ice slurry ingestion appeared to be the most beneficial per-cooling strategy. Previous studies primarily focused on different cooling techniques or a different timing of cooling, while less attention was given to the cooled body surface, the duration of cooling, the temperature of the intervention (cooling power), and the most suitable body location for cooling.

Cooled body surface

Our findings that mixed method or the single use of cold water immersion pre-cooling is most effective to improve exercise performance suggest that cooling of a large surface of the body is more effective than cooling local body parts. Accordingly, Minett *et al.* demonstrated that performance is affected by the dose of pre-cooling, in which a larger surface area coverage of cooling resulted in an increased exercise performance[29]. Higher cooling volumes may result in greater physiological perturbations and an attenuated thermoregulatory burden during exercise[29-31]. Furthermore, cooling of larger parts of the body is associated with a decreased perceptual strain, represented by a lower rate of perceived exertion and a lower thermal sensation during exercise[29]. These results suggest a dose-response relationship between cooling volume (surface area) and ensuing physiological, perceptual and performance outcomes, with larger performance improvements following cooling of larger parts of the body.

Duration of cooling

The duration of pre-cooling can also impact on its effectiveness as a previous study demonstrated a lower physiological strain and an improved exercise performance after 20 min *versus* 10 min of mixed method pre-cooling[32]. This may implicate that longer duration cooling results in an improved maintenance of thermal control, with an associated preservation of neuromuscular force production and subsequent performance benefits. Therefore, longer duration pre-cooling may preserve the reduced skin temperature and thermal sensation, and may result in a similar absolute Tc despite of a greater workload[32]. This dose dependent response of pre-cooling duration suggests that creating a large heat storage capacity prior to exercise is of great importance to improve exercise performance. The larger heat removal with more extensive pre-cooling, reduces the sweat loss induced alterations in blood volume, which prevent cardiovascular drift and attenuate the increase in heart rate[33]. Importantly, until now only 1 study investigated the dose-response relationship of cooling duration and exercise performance, therefore, more insight into this topic is necessary.

Cooling power

Another factor that impact on the efficiency of a cooling is the cooling power of an intervention. In this thesis (*Chapter 7*) we demonstrated that wearing an evaporative cooling vest (~10°C) did not improve 5-km time trial performance. In contrast, using an ice-vest (<0°C) during exercise turned out to be effective in increasing the time to exhaustion[34, 35]. The absence of a performance benefit in our study might be explained by the relatively low cooling capacity of the evaporative cooling vest compared to an ice vest. A study by Bogerd *et al.* made a direct comparison between mild (evaporative cooling shirt, cooling power = 27.3±1.1 W/m^2) *versus* strong cooling (ice-vest, cooling power = 49.2±9.0 W/m^2)[35]. They found a similar cooling efficiency, but a larger performance increment following ice-vest cooling, which suggests that the cooling power influences the effectiveness of a cooling intervention. Moreover, our results

of *Chapter 5* suggest that aggressive cooling strategies with a high cooling power are most effective. This can be explained by the law of enthalpy of fusion, which states that ice possesses a significantly larger capacity to absorb heat than liquid water[36, 37]. Accordingly, more aggressive cooling techniques, typically depending on ice or substances with a temperature below zero, demonstrate a larger effect on Tc and/or exercise performance.

Location of cooling

In this thesis we showed that local cooling strategies are effective in improving exercise performance, in which a distinction can be made between external and internal local cooling strategies. In literature different parts of the body were used for the application of external local cooling, for example the neck region[38-40], hand palm[41, 42], torso[40], and legs[30]. Therefore, the question raises which location is most effective to lower thermal strain and improve exercise performance, when the cooled surface is exactly similar. Shvartz and colleagues suggested that cooling the neck region is more effective in reducing heat strain compared to cooling the same surface area of the chest[43]. This might be explained by the proximity of large blood vessels to the skin of the neck region. Furthermore, the head, neck and face have been established as regions with a high thermosensitivity, caused by a greater thermoreceptor density[39, 44]. Therefore, locations on the body with a high density of thermoreceptors seemed to be the most efficient locations on the body for the application of cooling strategies. In contrast, based on our results of *Chapter 5 & 6* we may conclude that hand palm cooling (effect size = 0.63, n=2 studies) is more effective compared to neck collar cooling (effect size = 0.37, n=6 studies). Next to a high thermosensitivity, the face and neck region also have a high sensitivity for sudomotor control. As a consequence, local cooling may result in a reduced local sweat response, which may exacerbate heat storage. The hand palm region consists of a high surface area:volume ratio, large reserves for skin blood flow, and generally lower thermosensitivities[44]. Thereby, hand palm cooling may have little impact on perceptual thermal strain, but is more effective in reducing heat storage and improving performance[44]. Additionally, Daanen and colleagues demonstrated that the heat strain and gross efficiency did not differ when pre-cooling was applied to the body part with the exercising muscles or tissues elsewhere in the body[31]. This suggests that local cooling does not have to be applied on active body parts to be beneficial.

Next to the external cooling strategies, local cooling can also be applied internally. We demonstrated that internal cooling (cold water or ice slurry ingestion) is the most effective per-cooling strategy. Internal cooling strategies have been associated with a decreased Tc, an increased heat storage capacity and a declined cardiovascular strain[45]. The ingestion of cold water or ice slurry might result in a decreased perception of thermal strain and inhibited afferent feedback from the stomach thermoreceptors[45, 46]. As a result, the anticipatory reduction in skeletal muscle activation to prevent damage to the tissue is decreased[16, 46]. Therefore, internal cooling is very effective and easily applicable as per-cooling strategy.

In short, since athletes of different types of sports have to deal with different circumstances, such as environmental conditions, clothing regulations, exercise durations and exercise intensities, further optimization of cooling strategies and the development of personalized cooling strategies are of great importance. Based on our results we would like to suggest that mixed method cooling strategies with an 'aggressive' approach and affecting a large body surface is most effective in improving exercise performance. Internal cooling strategies can easily be applied during exercise and can therefore be used in combination with aggressive pre-cooling techniques.

Implementation of cooling strategies in elite athletes

A previous cohort study preceding the International Association of Athletics Federation (IAAF) World Championships of Beijing 2015 demonstrated that 52% of the athletes used at least one pre-cooling strategy, while 89% of the athletes used at least one recovery strategy (*i.e.* post-cooling, stretching, massage, active recovery)[47]. In contrast, none of them used per-cooling strategies, besides cold water ingestion to maintain hydration status, to improve exercise performance. The absence of per-cooling strategies could be the result of practical considerations of per-cooling, such as potential gastrointestinal discomfort with ice slurry ingestion, carrying extra weight during exercise with cooling packs or cooling vests, or simply not applicable during exercise (cold water immersion). On the other hand, it might be explained by ignorance of athletes about the beneficial effects of per-cooling. Future studies should therefore focus on applying per-cooling strategies in the normal routine of athletes during an exercise event in hot and humid conditions. Furthermore, current per-cooling strategies should be improved or new strategies should be developed to reduce the practical limitations of per-cooling during an exercise event. Ideally, some light weight cooling material should be incorporated into the sports clothing of athletes, which enables powerful cooling of a large body surface without adding extra weight.

Dehydration

Besides the effects of cooling on Tc and exercise performance, cooling might also have an indirect effect on fluid balance. As a consequence of cooling, the thermal strain during exercise is lower, resulting in an attenuated need for evaporative heat loss. Therefore, cooling might lower the sweat rate and decrease the associated disturbances in fluid balance and the development of dehydration. The overall consensus in literature is that dehydration of ≥2% body mass loss represents a threshold at which endurance exercise performance becomes impaired[48, 49]. This threshold does apply at fixed exercise intensities in lab based conditions, but not for athletes performing in an outdoor exercise event[50]. In field-based conditions dehydration up to 4% of body mass seems not to degrade exercise performance[50, 51]. The exact threshold for performance decrements following dehydration is therefore not yet known and will likely differ among individuals. However, the continuously monitoring of the fluid balance during exercise might help athletes to apply efficient rehydration strategies, which can attenuate the negative impact on thermoregulatory burden and exercise performance.

With current technology, we are able to measure the exercise intensity and Tc continuously during exercise, while an accurate, continuous and real time measurement of the hydration status during exercise is lacking. Current methods to measure the hydration status (*i.e.* body mass changes, plasma volume change, plasma and urine osmolality, plasma sodium concentration, and urine specific gravity) are not very accurate, but suitable to give an indication of an athlete's hydration status prior to or after exercise[52, 53]. However, these techniques cannot be used during exercise in field based settings, since athletes need to temporarily stop exercise to perform a measurement. Further development is therefore necessary to enable real time feedback with respect to the hydration status during exercise. However, a continuous measurement of the hydration status is difficult, since fluid balance regulation is a dynamic process of fluid exchange between the intracellular, interstitial and intravascular fluid compartment[54, 55]. A potential option for real time measurement of the fluid balance could be near infrared absorption spectroscopy[56]. Water absorbs much light when the wavelength is close to the infrared spectrum. When infrared light goes through a tissue, the infrared light will partly be absorbed by various components in the tissue[56]. For example, when infrared light is sent through a millimeter of pure water, 96% of the light will be absorbed, whereas only 80% of the light will be absorbed when the infrared light goes through tissue that consists for 50% of water[56]. Therefore, based on the absorption rate the water concentration could potentially be measured. However, to measure the hydration status using infrared absorption spectroscopy, the body tissue may not contain other substances that absorb light of the specific infrared wavelength, since this might influence the accurateness of the measurement. Therefore, more research into this measurement method is necessary to determine the usability, accuracy and validity.

Role of the kidneys

The increased fluid loss and level of dehydration following exercise, stimulate the kidneys to preserve and reabsorb water and sodium chloride by upregulating the vasopressin (AVP) secretion and activating renin-angiotensin-aldosterone system (RAAS)[57, 58]. The kidneys are therefore an important contributor for the maintenance of fluid balance during exercise. However, the increased energy-demanding renal sodium uptake and the reduced renal perfusion with excessive dehydration may induce ischemic kidney stress[59], which might impact on kidney function and the development of kidney injury. Furthermore, exercise can increase kidney injury biomarkers as a consequence of increases in Tc, increases in protein excretion and inflammation[60-62]. Kidney Injury Molecule-1 (KIM1) and Neutrophil Gelatinase-Associated Lipocalin (NGAL) are used as early biomarkers to detect kidney injury[63, 64], and increased levels were found after prolonged exercise[60, 65].

As shown in this thesis, no further increase in kidney injury biomarkers was found after repetitive exercise. Moreover, the urinary KIM1 and NGAL levels were well below the clinical thresholds for kidney injury and none of the subjects reported kidney-related health issues

after the exercise. Epidemiological studies demonstrated that an active lifestyle is associated with a reduced risk to develop chronic kidney disease[66-68]. An active lifestyle protects against chronic kidney disease by reducing the risk on hypertension and diabetes, which are important independent risk factors for kidney disease[66]. Furthermore, it is hypothesized that regular exercise in individuals with chronic kidney disease may slow the progression of kidney disease[69-71], and lower levels of physical activity were associated with an increased mortality among people with chronic kidney disease[72]. So although exercise and dehydration resulted in kidney stress, our studies did not reveal exercise-induced kidney damage. We hypothesize that the increase in kidney injury biomarkers during prolonged exercise might be a physiological response of the kidneys to obtain beneficial adaptations and profit from the health benefits of an active lifestyle.

Translation from athletes to clinical settings and the general population

Within this thesis we mainly focused on the thermoregulatory and fluid balance responses to exercise. Indeed, athletes are prone to develop high core body temperatures as a result of the markedly increased exercise-induced metabolic heat production. Although common in athletes, hyperthermia and heat-related illnesses also affect elderly, patients with predisposing medical conditions and patients taking different types of medication[73]. Moreover, in high demanding circumstances with a high ambient temperature, heat stroke can occur without exercising (classical or non-exertional heat stroke) and might even result in severe heat-related illnesses or death[73, 74]. Therefore, the thermoregulatory responses and potential benefits of cooling strategies in resting conditions in a thermoregulatory vulnerable population might also be of great importance.

Global warming

Global warming has been described as an increase in the average temperature of the Earth's atmosphere either by human industry and agriculture or by natural causes. According to ongoing measurements of NASA, the average global temperature on Earth has increased ~1°C since 1880, in which two-thirds of the increase has occurred since 1975 with a rate of 0.15-0.20°C per decade (Figure 4)[75-77]. Moreover, 16 of the 17 warmest years have occurred since 2001, with 2016 as the warmest on record[75]. Extreme heat waves are a significant public health problem that will be exacerbated by urbanization and an aging population[78, 79]. In fact, extreme heat waves are the primary cause of weather-related human mortality, which is responsible for more deaths annually than hurricanes, earthquakes, lightning, tornadoes and floods combined[78, 80]. As a result of global warming, the frequency and intensity of heat waves is expected to increase worldwide[78, 79]. Although heat waves have been relatively uncommon in temperate climates this is likely to change with global warming[81].

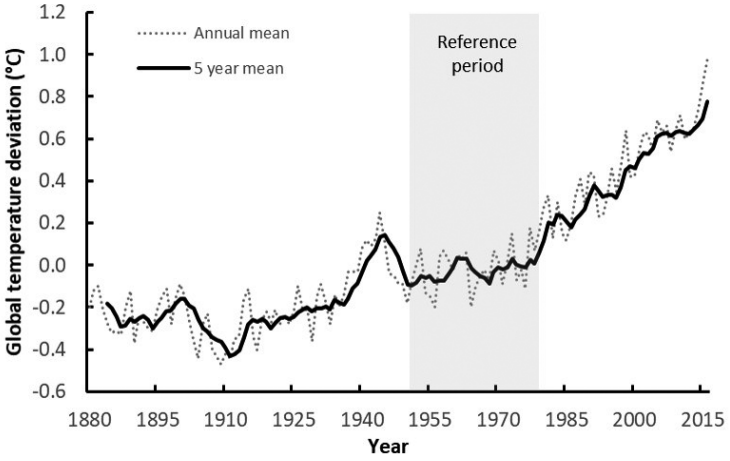


Figure 4. Change in global surface temperature compared to the 1951-1980 average temperatures (three-decade period to define reference temperature). The first global temperature recordings were performed in 1880. The grey dotted line represents the annual mean, while the black line represents a 5-year mean.

For example, the 2003 severe heat wave in France, where ~15.000 people died of heat stroke, was more severe and inflicted substantially more mortality than a typical heat wave in temperate climates[81]. Therefore, the thermoregulatory responses of vulnerable people during heat waves and interventions to counteract the increase in Tc are of significant importance. In athletes we found that cooling is effective in decreasing the core and skin temperature during exercise, which might suggest that cooling strategies could also be useful during heat waves to prevent vulnerable populations for heat-related illnesses.

Populations at risk to develop classical heat stroke

Advanced age is one of the most significant risk factors for heat-related death[78, 82]. Elderly are known to have a diminished thermoregulatory and physiological heat-adaptation ability[18]. Moreover, advanced age is associated with a lower baseline Tc, a reduced skin vasodilatory capacity, a less effective sweat response, and a decreased sensitivity of the thermal receptors[18, 83]. As a result, elderly have a reduced heat loss capacity and demonstrated a delayed response to heat gain. The decreased thermal sensitivity with age occurs for both warm and cold sensitivities, but the decrement in the perception of warmth is more pronounced[83]. Next to the physiological adaptations with aging, elderly are more likely to live alone, have reduced social contacts and experience poor health, which are individual risk factors for heat-related death[82, 84]. Furthermore, the rates of heat-related mortality and morbidity are high in chronically ill individuals, particularly those with cardiovascular, respiratory or renal diseases[84, 85]. The higher risk in these groups may be explained by a less efficient thermoregulation[85]. Moreover, the inadequate thermoregulation

may occur when too much blood is diverted from the vital organs to the skin's surface, which results in an increased stress on the heart, lungs and kidneys and might trigger heat-related mortality[74]. Furthermore, heat exposure can contribute to exacerbation of the pre-existing chronical diseases, which can result in heat-related mortality as well[84, 85].

Most of the knowledge regarding the thermoregulatory adaptations with aging is derived from cross-sectional observations between cohorts of young (<30 years) and older humans (usually >60 years). Accordingly, it is unknown whether thermoregulation deteriorates further with aging or plateaus at some point. Therefore, in *Chapter 9* we examined the thermoregulatory responses to prolonged walking exercise of elderly, in which we compare a group of sexagenarians (60±1 year) and octogenarians (81±2 year). We found a larger exercise-induced increase in Tc in octogenarians compared to sexagenarians. This suggests that the thermoregulatory responses progressively deteriorate with advancing age and thereby increases the risk on heat-related death during a heat wave. As life expectancy rises, the proportion of the population above a certain age rises as well, which is known as population aging. Moreover, in 2017 there are an estimated 962 million people aged 60 years or over in the world, which is 13% of the global population[86]. This group of people is growing at a rate of ~3% per year, in which the number of people aged 60 years or over is projected to be 1.4 billion (16.5%) in 2030 and 2.1 billion in 2050 (21.6%)[86]. Furthermore, the number of people aged 80 years or over is growing even faster, from 125 million in 2015 to 434 million in 2050[87]. Therefore, the combination of an expected worldwide increase in frequency and intensity of heat waves with an aging population may increase the risk on and incidence of classical heat stroke or heat-related death.

For that purpose it is important that the general practice in nursing homes for elderly acts adequately to the high demanding conditions during a heat wave. A continuous monitoring of Tc might be very useful to determine the individual thermal strain and prevent the development of classical heat stroke. The ingestible telemetric temperature capsule is very suitable for this purpose, since it is very accurate, relatively non-invasive and easy in use. Moreover, as a result of the longer transit time with aging, the temperature capsule might stay in the body for a longer period, which enables measurement on multiple days with a single capsule[88]. Furthermore, public health recommendations for effective heat management strategies are absolutely essential to minimize heat-related mortality. Electric fans provide a low-cost cooling intervention, although their effectiveness in elderly is debatable[82, 89]. Fan use above ambient temperatures of ~35°C in elderly is discouraged, since it accelerated heat gain and dehydration[82, 89]. Therefore, other strategies have to be implemented to daily practice in nursing homes for elderly to decrease the number of heat-related deaths during heat waves.

In this thesis we demonstrated that pre-cooling strategies effectively lower Tc in resting conditions, and may therefore reduce the risk to develop classical heat stroke. Cold water

immersion has been demonstrated as most effective single pre-cooling strategy, however, this is not very suitable for daily practice and should only be used in emergency situations to rapidly cool down individuals suffering from heat stroke. More practical pre-cooling strategies could be a cooling vests or cooling packs. A previous study demonstrated that personal cooling with a cooling vest of phase change material can be used to improve thermal comfort and decrease skin temperature of office workers (27±2 years) working in an ambient temperature of 34°C without air conditioning, which suggests that the cooling intervention may be used for vulnerable groups when confronted with heat waves[90]. Furthermore, adding a cold water spray to the electric fan might increase the effectiveness as well, by stimulating heat loss. Internal cooling using cold water or ice slurry ingestion might be even better, since it combines pre-cooling with rehydration. In an ideal situation multiple cooling strategies will be combined to prevent heat-related disorders in vulnerable groups during a heat wave. Conclusively, cooling strategies are not only effective in improving exercise performance in athletes, but can also be used to decrease the thermal strain of vulnerable people in hot and humid conditions.

Clinical implications

This thesis showed that prolonged exercise is associated with increased levels of kidney stress, which are not harmful for exercising individuals. Furthermore, exercise is associated with an increase in Tc, which can be measured accurately using ingestible telemetric temperature capsules. Advanced aging is associated with a greater increase in Tc and elderly are therefore at risk to develop exertional hyperthermia, particularly in high demanding circumstances such as exercise and heat waves. We demonstrated that pre-, per- and post-cooling are effective strategies to reduce the thermoregulatory burden of exercise and improve exercise performance in the heat. Furthermore, it is hypothesized that cooling may reduce heat-related morbidity and mortality in vulnerable groups during a heat wave.

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Chapter 13

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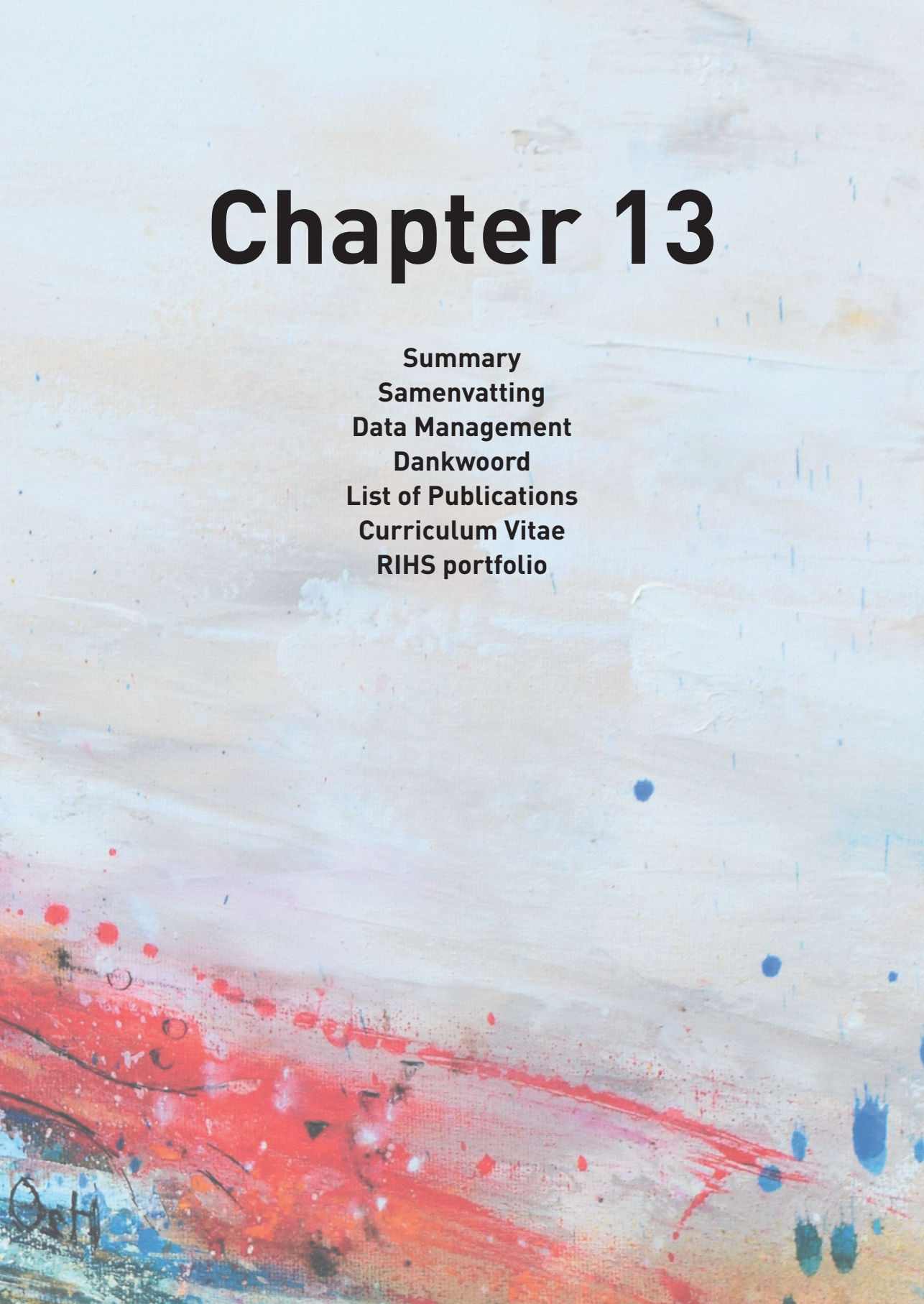

SUMMARY

In **Chapter 1** we provided a historical overview of the measurement of core body temperature (Tc) and we introduced the general concepts of the human thermoregulation and fluid balance control. Exercise is associated with the development of an increased core body temperature (hyperthermia) and dehydration. Both hyperthermia and dehydration may negatively impact on exercise performance and the development of heat-related illnesses, which emphasizes the importance of interventions to attenuate the exercise-induced increase in Tc. Therefore, the general aim of this thesis was to evaluate the thermoregulatory and fluid balance responses to exercise in young and elderly individuals, using State-of-the-Art equipment. Second, we aimed to get more insight into cooling strategies to determine the most beneficial cooling strategy to improve exercise performance in the heat.

An accurate measurement of Tc is of great importance to quantify the thermal strain in rest and during exercise. Ingestible telemetric temperature capsules have been described as a valid surrogate marker for Tc. In **Chapter 2** we provided a detailed prescription of a measurement protocol using the CorTemp ingestible temperature capsule. Furthermore, we identified important factors that should be addressed, while using an ingestible temperature capsule to measure Tc in lab- and field based settings. Current available capsule systems are expensive and have restrictions related to the battery life time and expiry. Therefore, we assessed the validity and test-retest reliability of a novel ingestible temperature capsule (myTemp) in well-controlled ex-vivo circumstances in **Chapter 3**. A total of n=15 capsules were tested twice in a highly temperature controlled water bath, in which the water temperature was gradually increased from 34°C to 44°C. Based on the low systemic bias, narrow 95% limits of agreement, high intraclass correlation coefficient and a low standard error of the mean, we concluded that the myTemp ingestible temperature capsule is a valid and reliable technique to measure (water) temperature. Subsequently, we compared the validity, reliability and inertia characteristics of four different temperature capsule systems (n=10 capsules per system) in **Chapter 4**, using an ex-vivo water bath. We demonstrated that all capsule systems are valid and reliable to measure (water) temperature. The best test–retest reliability was found for the myTemp and VitalSense system, whereas CorTemp and e-Celsius demonstrated a small, but negligible, systematic bias. Furthermore, the VitalSense system showed the slowest response to increases in water bath temperature, whereas the other systems had a comparable time delay. These findings may implicate that ingestible temperature capsules are eligible to measure Tc.

Strategies to reduce the thermal strain prior to (pre-cooling), during (per-cooling) and directly after exercise (post-cooling) are able to improve exercise performance and enhance recovery from exercise. In **Chapter 5** we performed a meta-analysis, in which we demonstrated that pre- and per-cooling are equally effective in improving exercise performance in the heat (ambient

temperature ≥30°C). Moreover, we revealed that a combination of cooling techniques (for pre-cooling) or ice vests (for per-cooling) is most effective in improving exercise performance. Pre-cooling was also effective in reducing the maximum Tc, whereas no difference in Tc was found after per-cooling. Additionally, no correlation was found between maximal Tc and improvement in exercise performance, which suggests that a lower Tc at the end of exercise does not necessarily improve exercise performance in the heat.

In **Chapter 6** we provided a comprehensive overview of current scientific knowledge in the field of pre-, per- and post-cooling, in which we discussed the effectiveness of cooling interventions, the underlying physiological mechanisms and the practical regulations regarding the use of cooling techniques. Our findings suggest that pre-cooling, per-cooling and a combination of both are equally effective in improving exercise performance. The beneficial effects of pre- and per-cooling can be explained by thermoregulatory as well as cardiovascular and metabolic mechanisms, while the beneficial effects of post-cooling can be explained by faster recovery of thermoregulatory and cardiovascular strain and a reduced inflammatory response to exercise. In short, any opportunity to reduce thermal strain prior to, during or after exercise is effective to improve exercise performance and recovery from a stressful bout of exercise.

The effects of wearing an evaporative cooling vest during a 5-km running time trial on exercise performance and thermoregulatory responses is examined in **Chapter 7**. A total of 10 well-trained subjects were included and they completed a 5-km time trial during three study visits (familiarization, cooling and control session). We showed that wearing an evaporative cooling vest during exercise did not improve 5-km time trial performance or attenuate the increase in Tc in moderate ambient conditions (25°C and 55% relative humidity), but did improve thermal comfort during exercise. These findings suggest that although the cooling vest did not enhance exercise performance, it might be comfortable during practice.

Individuals with spinal cord injury are known to have a reduced thermoregulatory function below the level of the lesion. Therefore, the effects of wearing an evaporative cooling vest on the Tc response of individuals with thoracic spinal cord injury during submaximal exercise was examined in **Chapter 8**. We included n=10 subjects with a thoracic lesion and they performed two 45-minute exercise bouts at 50% of maximal workload in moderate ambient conditions (25°C). Wearing a cooling vest effectively lowered skin temperature, perception of thermal sensation and increased the core-to-skin temperature gradient, but cooling was not effective in limiting or delaying the increase in Tc. Therefore, an evaporative cooling vest might be comfortable for individuals with spinal cord injury during exercise in moderate ambient conditions despite no impact on Tc was observed.

The impaired thermoregulatory and fluid balance responses to exercise in older individuals are well established, however it was not yet known whether these responses further deteriorate with advanced aging. Therefore, in **Chapter 9** we compared the thermoregulatory and fluid balance responses between sexagenarians (60±1 year, n=40) *versus* octogenarians (81±2 year, n=39). All subjects participated in the first day of the Nijmegen Four Days Marches and walked 30 km at a self-selected pace. We found a larger exercise-induced increase in Tc in octogenarians compared to sexagenarians. Furthermore, octogenarians reported a lower fluid intake and body mass loss compared to sexagenarians, whereas no differences traditional fluid balance parameters were found. Our findings suggest that the thermoregulatory control declines with advanced aging, while the fluid balance control did not deteriorate with aging.

Literature showed that completing a (ultra) marathon results in increased urinary levels of Kidney Injury Molecule-1 (KIM1) and Neutrophil Gelatinase-Associated Lipocalin (NGAL), and suggested an exercise-induced development of acute kidney injury. In **Chapter 10** we examined the effects of a single *versus* repetitive bouts of prolonged moderate intensity exercise on markers for kidney injury. We included n=60 subjects participating in the Nijmegen Four Days marches, who walked 30, 40 or 50 km at a self-selected pace for three consecutive days. We found that a single bout of prolonged exercise did not impact on KIM1, but did increase urinary NGAL concentration. Furthermore, no differences in kidney response to a single *versus* repetitive bouts of prolonged exercise were found, which suggests that there is no cumulative effect of exercise on kidney injury biomarkers.

Subsequently, in **Chapter 11** we aimed to directly compare the effects of acute *versus* prolonged exercise on kidney function and kidney injury biomarkers in well-controlled laboratory circumstances in healthy male adults. A total of n=35 healthy and young adults (23±3 years) were included and performed a submaximal exercise test at 80% of maximal heart rate until 3% dehydration. We found that acute exercise barely impacted on kidney function and injury, whereas prolonged exercise is associated with a decreased kidney function and increased level of kidney injury biomarkers. These findings suggest that the kidneys are well able to maintain kidney function during an acute bout of exercise, whereas the exercise-induced kidney stress alters kidney function and elevates biomarkers for kidney injury after prolonged exercise.

In **Chapter 12** the findings of the studies presented in this thesis were summarized and discussed. Additionally, we translated the relevance of our findings in athletes and exercise settings to clinical settings and the general population.

SAMENVATTING

Hoofdstuk 1 geeft een historisch overzicht van het meten van de lichaamstemperatuur. Daarnaast worden de algemene principes van de humane thermoregulatie en vochtbalans geïntroduceerd. Inspanning wordt geassocieerd met het ontwikkelen van een verhoogde lichaamstemperatuur (hyperthermie) en uitdroging (dehydratie). Zowel hyperthermie als dehydratie kunnen een negatief effect hebben op de sportprestatie en het ontwikkelen van hitte-gerelateerde aandoeningen. Dit benadrukt het belang van interventies om de stijging in lichaamstemperatuur tijdens inspanning te verminderen. Het doel van dit proefschrift was om de thermoregulatie en vochtbalans tijdens inspanning te onderzoeken met behulp van geavanceerde meettechnieken. Daarnaast wilden we meer inzicht krijgen in het gebruik van koel strategieën met als doel te bepalen welke koel strategie het meest effectief is voor het verbeteren van de sportprestatie in de hitte.

Een accurate meting van de lichaamstemperatuur is van groot belang voor het kwantificeren van de thermische belasting in rust en tijdens inspanning. Het gebruik van temperatuur capsules is beschreven als een betrouwbare methode om de lichaamstemperatuur te meten. In **Hoofdstuk 2** hebben we een gedetailleerde beschrijving gegeven voor het gebruik van de CorTemp temperatuur capsule. Tevens hebben we belangrijke factoren geïdentificeerd waar rekening mee gehouden dient te worden, wanneer de lichaamstemperatuur in lab/ en veldcondities gemeten wordt met een temperatuur capsule. Huidige beschikbare temperatuur capsules zijn duur en hebben beperkingen betreft de levensduur en houdbaarheid van de batterij. Daarom hebben we in **Hoofdstuk 3** de nauwkeurigheid en betrouwbaarheid van een nieuwe temperatuur capsule (myTemp) getest in gecontroleerde ex-vivo omstandigheden. Vijftien temperatuur capsules zijn twee keer getest in een zeer nauwkeurig temperatuur gecontroleerd waterbad, waarbij de water temperatuur stapsgewijs toenam van 34°C naar 44°C. Op basis van de lage systematische afwijking en sterke correlatie tussen waterbad en capsule temperatuur kunnen we concluderen dat de myTemp temperatuur capsule een nauwkeurige en betrouwbare techniek is om de (water) temperatuur te meten. Vervolgens hebben we in **Hoofdstuk** 4 de nauwkeurigheid, betrouwbaarheid en traagheid van vier verschillende systemen (10 capsules per systeem) met elkaar vergeleken gebruik makend van een waterbad. We vonden dat alle systemen nauwkeurig en betrouwbaar de (water) temperatuur kunnen meten. Het myTemp en VitalSense systeem hebben de beste betrouwbaarheid bij het herhaaldelijk meten, terwijl CorTemp en e-Celsius een kleine, maar verwaarloosbare, systematische afwijking lieten zien. Het VitalSense systeem reageert het traagst op een stijging van de temperatuur van het waterbad, terwijl de andere systemen een vergelijkbare vertraging hebben. Deze bevindingen tonen aan dat het gebruik van temperatuur capsules geschikt is om de lichaamstemperatuur te meten.

Het gebruik van interventies om de thermische belasting voorafgaand aan (pre-koeling), tijdens (per-koeling) en direct na inspanning (post-koeling) te verminderen, maken het mogelijk om

de sportprestatie en het herstel te bevorderen. In **Hoofdstuk 5** hebben we een meta-analyse uitgevoerd, waarin we laten zien dat pre- en per-koeling even effectief zijn voor het verbeteren van de sportprestatie in de hitte (omgevingstemperatuur ≥30°C). Verder hebben we aangetoond dat een combinatie van koel technieken (voor pre-koeling) of het gebruik van een ijs vest (voor per-koeling) het meest effectief is voor het verbeteren van sportprestatie. Pre-koeling was daarnaast ook effectief in het verlagen van de maximale lichaamstemperatuur, terwijl per-koeling geen effect heeft op de maximale lichaamstemperatuur. Verder vonden we geen correlatie tussen de lichaamstemperatuur en prestatieverbetering, wat suggereert dat een lagere temperatuur aan het eind van de inspanning niet noodzakelijkerwijs een prestatieverbetering betekent.

Hoofdstuk 6 geeft een uitgebreid overzicht van de huidige wetenschappelijke kennis op het gebied van pre-, per- en post-koeling, waarbij we de effectiviteit van koel strategieën, de onderliggende mechanismen en de praktische aspecten van het gebruik van koel strategieën bespreken. Onze bevindingen suggereren dat pre-koeling, per-koeling en een combinatie van beide even effectief zijn voor prestatieverbetering. De voordelige effecten van pre- en per-koeling kunnen verklaard worden door thermoregulatoire, cardiovasculaire en metabole mechanismen, terwijl de positieve invloed van post-koeling verklaard kan worden door een sneller herstel van de thermische en cardiovasculaire belasting en een verminderde inflammatoire response na inspanning. Kortom, iedere mogelijkheid om de thermische belasting voorafgaand aan, tijdens of na inspanning te verminderen, kan de sportprestatie verbeteren en het herstel na een stressvolle inspanning bevorderen.

Het effect van het dragen van een koelvest tijdens een 5 km tijdrit op de prestatie en thermoregulatie is onderzocht in **Hoofdstuk 7**. In totaal voltooiden 10 goed getrainde proefpersonen een 5 km tijdrit op de loopband gedurende 3 studie bezoeken (gewenning, koeling en controle sessie) aan de klimaatkamer van sportcentrum Papendal. We vonden dat het dragen van een koelvest tijdens inspanning geen invloed heeft op de 5 km tijdrit prestatie of de stijging in lichaamstemperatuur in gematigde omstandigheden (25°C en 55% luchtvochtigheid), maar wel een positief effect heeft op het thermische comfort tijdens inspanning. Deze bevindingen suggereren dat ondanks het koelvest de sportprestatie niet bevordert, het dragen comfortabel kan zijn tijdens trainingen.

Het is bekend dat individuen met een dwarslaesie een verminderde thermoregulatie hebben onder het niveau van de laesie. Daarom hebben we in **Hoofdstuk 8** de effecten van het dragen van een koelvest op de lichaamstemperatuur van individuen met een thoracale dwarslaesie onderzocht tijdens een sub maximale inspanning. Tien proefpersonen hebben twee keer een inspanningsprotocol van 45 minuten op 50% van de maximale belasting uitgevoerd in gematigde omgevingsomstandigheden (25°C). Het dragen van het koelvest verminderde de huidtemperatuur en het thermische comfort. Maar het koelvest was niet effectief in het beperken of vertragen van de stijging in temperatuur. Een koelvest kan daarom comfortabel zijn

voor dwarslaesie patiënten tijdens inspanning in gematigde omstandigheden, ondanks dat het geen invloed heeft op de lichaamstemperatuur.

Ouderen hebben een verminderde thermoregulatie en vochtbalans tijdens inspanning, echter het is onbekend of de thermoregulatie en vochtbalans blijven afnemen met verdere veroudering. Daarom hebben we in **Hoofdstuk 9** de thermoregulatie en vochtbalans van zestigjarigen (n=40) vergeleken met tachtigjarigen (n=36). Alle proefpersonen namen deel aan de Vierdaagse van Nijmegen en liepen 30 km op een zelf gekozen snelheid. We vonden een grotere stijging in lichaamstemperatuur in de tachtigjarigen in vergelijking met de zestigjarigen. Daarnaast rapporteerden de tachtigjarigen een lagere vocht inname en gewichtsafname, terwijl er geen verschillen werden gevonden in de traditionele vochtbalans uitkomstmaten gemeten in het bloed en de urine. Onze bevindingen suggereren dat de thermoregulatie afneemt met verdere veroudering, terwijl de vochtbalans niet veranderd met verdere veroudering.

Uit onderzoek is gebleken dat het volbrengen van een (ultra) marathon resulteert in verhoogde concentraties nierschademarkers (KIM1 en NGAL) en suggereert daardoor een inspannings-gerelateerde ontwikkeling van acute nierschade. In **Hoofdstuk 10** hebben we de effecten van een enkele ten opzichte van herhaaldelijke duurinspanning op een gematigde intensiteit op uitkomstmaten voor nierschade onderzocht. Zestig proefpersonen hebben deelgenomen aan de Vierdaagse van Nijmegen en liepen allen 30, 40 of 50 km op een zelf gekozen snelheid voor drie achtereenvolgende dagen. We vonden dat het voltooien van de eerste wandeldag geen invloed heeft op KIM1, maar wel voor een stijging van NGAL zorgt. Verder vonden we geen verschillen in nierschade tussen het voltooien van een enkele of drie achtereenvolgende wandeldagen, wat suggereert dat er geen cumulatief effect is van inspanning op nierschade.

Vervolgens hebben we in **Hoofdstuk 11** de effecten van acute en langdurige inspanning op nierfunctie en nierschade onderzocht in goed gecontroleerde omstandigheden in gezonde jonge mannen. In totaal hebben 35 proefpersonen een sub maximale inspanningstest uitgevoerd op 80% van de maximale hartslagfrequentie tot 3% afname in lichaamsgewicht (uitdroging). We vonden dat acute inspanning nauwelijks effect heeft op nierfunctie en de ontwikkeling van nierschade, terwijl langdurige inspanning de nierfunctie verminderd en uitkomstmaten voor nierschade verhoogd. Deze bevindingen suggereren dat de nieren goed in staat zijn om de functie te handhaven tijdens een acute inspanning, maar dat inspannings-geïnduceerde nier stress de nierfunctie verminderd en nierschade veroorzaakt na langdurige inspanning.

In **Hoofdstuk 12** worden de bevindingen van dit proefschrift samengevat en bediscussieerd. Daarnaast hebben we de relevantie van onze bevindingen in atleten tijdens inspanning vertaald naar klinische condities en de algemene populatie. Tevens worden de mogelijke gevolgen van de opwarming van de aarde (global warming) op de thermoregulatie besproken.

DATA MANAGEMENT

Appropriate data management is important for 1) knowledge discovery and innovation, 2) protecting scientific integrity and 3) preservation and reuse of data sets. The data used within this thesis are collected and stored according to the Findable, Accessible, Interoperable and Reusable (FAIR) principles[1]. During the first part of my PhD, the raw and processed data that were generated have been stored in encoded Microsoft Excel data files. All data files were stored at the local server of the Radboudumc, which was backed-up on daily basis to prevent data loss. In the second part of my PhD, the Castoredc data management system was introduced and implemented within the Radboudumc. Afterwards, the data was stored in Castoredc and an audit trail was used to provide documentary evidence of the activities that have affected the original data. This thesis is primarily based on results of human studies, which were conducted in accordance with the principles of the Declaration of Helsinki. Additionally, a local Medical Ethics Committee approved the study protocols, including a data management plan. All subjects were well informed about the study using an information package and all subjects gave written informed consent prior to participation in the study. All study procedures were monitored by an independent researcher according to the protocol compiled by the departments of Physiology and Intensive Care of the Radboudumc. The privacy of subjects is guaranteed due to anonymization of data using a unique and untraceable individual subject code. In all data files and case report forms the individual subject code is used, which allows us to share the data if necessary. The encryption key was only available for the research team. The raw and processed data sets are stored at the department of Physiology and will be available for further analyses for at least 15 years. In order to ensure that the data is generally accessible and interoperable, all file names and data, which are used to produce the final results, were documented using applicable language for knowledge representation. Furthermore, the data generated and analyzed in this thesis is on request available from the associated corresponding authors.

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DANKWOORD

Het is zondag. Ik bereid me voor op de voetbalwedstrijd van vanmiddag, maar eerst nog even mijn dankwoord schrijven. Niet het meest eenvoudige, maar wellicht wel het belangrijkste en meest gelezen onderdeel van het proefschrift. Zo'n 4,5 jaar geleden begon ik met een promotietraject met als doel het ontwikkelen van een nieuwe sensor om de vochtbalans continue te kunnen monitoren tijdens inspanning. Mijn promotie is voorbij gevlogen. Graag wil ik hier iedereen bedanken die, direct of indirect, hebben bijgedragen aan dit proefschrift en mijn ontwikkeling als persoon en onderzoeker.

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Thijs, jij hebt me, zo'n zes jaar geleden, enthousiast gemaakt voor de wetenschap. In de afgelopen jaren heb ik ontzettend veel van je geleerd. Ik bewonder je kwaliteit om altijd in oplossingen te denken, te allen tijde positief te blijven en overal kansen en uitdagingen in te zien. Vanaf het begin heb je heel erg veel vertrouwen in me gehad. Ieder jaar kreeg ik meer verantwoordelijkheid binnen 'jouw' vierdaagse onderzoek. We hebben de afgelopen jaren ontzettend prettig samengewerkt, maar ook veel leuke dingen gedaan! Een wedstrijdje van het Nederlands elftal kijken in de businessclass, ACSM congressen in Boston en Denver, geproost op publicaties of een geslaagd vierdaagse onderzoek en uit het wiel gereden worden op de Oude Holleweg. Naast copromotor, collega en kamergenoot, ben ik je ook als vriend gaan zien en kan ik alles met je bespreken. Thijs, heel erg bedankt voor onze samenwerking. Mede dankzij ons teamwork hebben we Thermo Nijmegen op de kaart gezet. Hopelijk gaan er in de toekomst nog mooie projecten volgen.

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Ton, Alan, Etienne, Paul & Paul. In oktober 2013 zijn we gestart met een gezamenlijk project, waarin we een nieuwe sensor wilden ontwikkelen. Helaas bleek dit ingewikkelder dan gedacht. Toch wil ik jullie hartelijk danken voor de prettige samenwerking en veel succes met jullie carrières. **Alan**, as you can see I assume you can understand Dutch in the meanwhile. If not, thanks a lot for all the pilot measurements and discussions we had about the hydration sensor.

Natuurlijk ben ik ook alle proefpersonen ontzettend dankbaar. In totaal hebben er 252 proefpersonen vrijwillig en zonder eigen belang deelgenomen aan alle metingen en inspanningen noodzakelijk voor het onderzoek. Of het nu ging om een maximale inspanningstest, een uitdrogingsprotocol of metingen na afloop van 50 km wandelen, jullie stonden overal voor open. Speciale dank gaat uit naar onze 'veteranen', de groep Vierdaagse wandelaars die vanaf 2007 ieder jaar hebben deelgenomen aan het onderzoek.

Naast mijn promotieteam heb ik de afgelopen jaren het geluk gehad samen te mogen werken met een leuke, gezellige en vooral ook sportieve groep collega's. Een biertje drinken in de aesculaaf, een wandeling maken in de pauze of ontsnappen uit een escape room, altijd was het gezellig. Maar ook fanatiek meedoen aan de Carréloop of Radboud Sports, het invullen van een SQUASH vragenlijst om de activiteitenscore van iedereen te berekenen of zelfs het uitvoeren van een maximale fietstest om te kijken wie de hoogste VO_2 max waarde heeft. Leuk om onderdeel te zijn van zo'n gezellig team! Bij dezen wil ik me nog één keer excuseren dat ik ~~BIJNA~~ altijd moest voetballen.

Bregina, jij bent de vrolijke noot van de afdeling. Altijd in voor een lach, een gezellig gesprek of het geven van je mening. Door jouw aanwezigheid wordt het leven van veel PhD's een stuk eenvoudiger. Heel erg bedankt voor de fijne samenwerking en natuurlijk voor het ontwerpen van de mooie kaft van dit proefschrift. **Dick**, tijdens mijn stage zag ik je vooral als de FMD-koning en het publicatiekanon. Later leerde ik je beter kennen. Bedankt voor alle nuttige tips en suggesties! **Silvie**, ontzettend knap hoe jij jezelf binnen hebt weten te werken op de afdeling. Altijd kritisch, scherp en gestructureerd. Veel succes met je nieuwe uitdaging in Wageningen, gelukkig blijf je nog aan de afdeling verbonden. **Milène**, bedankt voor onze samenwerking en je nuchtere blik op het onderzoek. Gelukkig heb ik je kunnen overtuigen dat thermoregulatie een ontzettend interessant onderwerp is. **Pascale**, bedankt voor al je hulp en het (soms nagenoeg onmogelijk) plannen van een afspraken met Maria.

Rebecca, als grondleggers van room paradise hebben we een mooie basis gelegd voor onze promoties. Van hard werken en publiceren naar voetjes op de verwarming en discussiëren over van alles en nog wat. Ik heb ontzettend veel respect je en voor de manier waarop jij al die nevenactiviteiten kunt combineren met promoveren, klinisch werk en mama zijn. Die gezamenlijke publicatie in de kerstspecial van BMJ moet er toch echt een keer gaan komen. Daaaaggggg! **Dominique,** het derde bemanningslid van room paradise. Door jouw aanwezigheid werd het nog gezelliger! Jij begrijpt als geen ander hoe leuk het is om in een dorp te wonen. Do, bedankt voor de gezelligheid, suggesties, hulp bij mijn projecten en het opstellen van belangrijke regels; I) gooi nooit een bananenschil in onze prullenbak en II) vergeet geen zesjes te maken. Gelukkig word je harde werken beloond en krijg je een prachtig proefschrift. Je bent een topper! **Lando**, allereerst wil ik je hartelijk danken voor je taalkundige opvoeding. Zonder jou was het nog slechter gesteld met mijn ABN. Maar gelukkig heb ik de weegschaal bij me! Bedankt voor al je hulp tijdens de Vierdaagse, je genoot er zichtbaar van. Veel succes met je promotie en veel geluk in jullie nieuwe huisje. We moeten snel weer een potje tafeltennissen. **Cindy**, het meest recente lid van room paradise. Een frisse en gezonde wind op de afdeling. Veel succes met je project en je uitdaging om een PhD traject te volbrengen op drie verschillende locaties.

Martijn, van samen stage lopen op de afdeling fysiologie naar nagenoeg tegelijk beginnen met een promotie op dezelfde afdeling. Jouw manier van werken en data presenteren is altijd een voorbeeld voor me geweest. Als ik nog advies nodig heb over ICT, fotografie en reizen met de trein, dan weet ik je te vinden. Heel veel succes met je nieuwe uitdaging bij VGZ. **Anke,** zo'n 10 jaar geleden zijn we begonnen aan dezelfde studie, maar ik leerde je pas echt kennen tijdens onze stage bij revalidatie. Vrij snel daarna kwamen we bij fysiologie terecht, om daar vervolgens niet meer weg te gaan. Ontzettend knap hoe jij het zo snel voor elkaar kreeg om het onderwijs meester te worden. Jij bent een aanwinst voor de universiteit. Veel geluk samen met Dennis. **Hugo**, held! Ik heb nog nooit iemand zo hard van start zien gaan tijdens de Carréloop en dat heb je geweten ook. Jij staat altijd klaar voor anderen en werkt ontzettend hard! Succes met het afronden van je promotie. **Yvonne,** jij weet als geen ander hoe je het voor elkaar moet krijgen om meer dan 5 studenten effectief voor je te laten werken. Bedankt dat jij en Anouk me altijd wisten te vinden als Ajax weer eens verloor. En wat hebben we, samen met Vincent en Esmee, een mooie tijd gehad in de Rocky Mountains! Een ervaring om nooit te vergeten. **Esmee**, jij weet precies wat je wilt en hoe je dit wilt bereiken. Met jouw kritische werkhouding en doorzettingsvermogen moet het zeker goed komen met jou promotie. P.S. denk aan je Kardashian-index! **Vincent,** als er iemand hard werkt dan ben jij het wel. Jij vindt alles interessant en ik sta versteld, zegge, van jou parate kennis. Bewonderenswaardig hoe jij het voor elkaar krijgt om zoveel grote projecten gelijktijdig te draaien. Je bent een aanwinst voor de afdeling en de wetenschap. Vergeet vooral niet te genieten, je promoveert (waarschijnlijk) maar een keer! **Eline,** door jou lijkt onderzoek doen heel eenvoudig. De rust, structuur en accuraatheid waarmee jij je onderzoek uitvoert zijn een perfect voorbeeld voor

iedere onderzoeker. Succes met de laatste loodjes van je studie en geniet van jullie nieuwe woning in Arnhem.

Lisa, Thijs, Bram, Geert, Carlijn en Malou, als nieuwe lichting hebben jullie nog een mooie tijd voor je. Veel succes met jullie projecten. Speciaal respect ook voor 'The Man Cave', hopelijk kunnen jullie alle doelstellingen tijdens jullie promoties bewerkstelligen. **Joep**, heel veel succes met het opzetten van het human performance lab. Een mooie uitdaging waar we hopelijk in de toekomst veel van kunnen genieten.

TimWasEenKoning, door jou ben ik, tijdens een korte stage in het 1e jaar, voor het eerst in aanraking gekomen met de afdeling fysiologie. Je stelde jezelf voor als geprogrammeerde aap die volledig tot onze dienst stond! Zo'n vier jaar later werden we collega's. **Nathalie, Joost en Matthijs**, als ervaren PhD's hebben jullie mij in mijn eerste fase een prachtig voorbeeld gegeven van hoe het moet. **Piet, Leonie, Thalijn, Femke, Linda, Jos en Elvira**, ook jullie zou ik willen bedanken. Door al jullie gezelligheid en wijze woorden heb ik een ontzettend mooie tijd gehad. **Marian**, bedankt voor de flauwe grappen, het schoonhouden van mijn werkplek en je bijdrage aan het materiaal voor het vierdaagse onderzoek. **Tjarda**, bedankt voor je inzet en hulp tijdens het vierdaagse onderzoek. Als chef-prikpost heb jij bij heel veel wandelaars bloed afgenomen. Natuurlijk wil ik ook alle onderzoekers bedanken voor de waardevolle bijdrage tijdens de metingen op de Wedren.

Alle collega's van de HAN, en in het bijzonder Joris en Jan-Willem, bedankt dat jullie mij de mogelijkheid hebben gegeven om onderdeel te zijn van jullie team. Ik heb me zeer welkom gevoeld en erg prettig met jullie samengewerkt.

Tijdens mijn promotie heb ik verschillende studenten mogen begeleiden. **Stefan, Hai, Paul, Niek, Richard, Jan, Arnoud, Anouk, Adriaan, Jasmijn, Daan, Nitya, Yannick, Michelle en Iris**, bedankt voor jullie waardevolle bijdrage aan mijn onderzoek. Veel succes met jullie toekomstige carrières.

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Next up, de **Roze olifantjes (#FFC0CB)**. Op zondag 17 augustus 2008 begonnen we aan een nieuw avontuur, studeren in Nijmegen beginnend met de introductie. Dankzij jullie is mijn studententijd voorbij gevlogen. Een potje hartenjagen in de kantine in plaats van ZSO´s maken,

biertjes drinken in de aesculaaf, gezellig ouwehoeren en de vele weekendjes weg. Ik ben ontzettend trots op jullie!

Team Erik, mijn paranimfen. **Erik Stroeken** met twee k's, één in Erik en één in Stroeken. Zo stelde jij jezelf voor tijdens de intro en dit was direct een voorbeeld van jou karakter. Altijd in voor een grap of een woordspeling. Nadat ik jou mocht toespreken na het behalen van je master diploma, vind ik het een enorme eer dat jij vandaag aan mijn zijde staat. Als healthcare consultant heb jij je plek gevonden. Ik wens je een fantastische carrière en mooie toekomst samen met Pleun! **Erik Dortmans**, carpool collega. We hebben er beide een handje van om te laat te komen. Jij meestal in de ochtend en ik 's middags na het werk. En wat hebben we het druk onderweg. Het raden van titel en artiest van de muziek op de radio, observeren van geiten en een witte suzuki swift op de route, verbeteren van onze topografische kennis, en het bespreken van belangrijke werk, weekend en voetbal gerelateerde zaken. Dit zijn slechts een aantal voorbeelden van onze activiteiten. Binnenkort zal je het voor 8 maanden alleen moeten doen, maar daar heb ik alle vertrouwen in! Veel geluk samen met Manon, maak er een mooie bruiloft van!

Pap en mam, ik wil jullie bedanken voor alles. Jullie staan altijd voor me klaar en zijn altijd geïnteresseerd in de dingen die ik doe. Jullie hebben me gevormd tot wie ik nu ben. Zonder jullie vertrouwen en onvoorwaardelijke steun was dit alles niet mogelijk geweest! Dit proefschrift is ook voor jullie. **Luuk en Thieu,** bedankt voor de gezelligheid, de gebruikelijke strijd tussen Ajax, PSV en Feyenoord, en jullie fanatisme, want ik ben niet de enige die altijd wil winnen. Luuk en Donna, veel geluk in jullie huisje en Thieu en Didi, houden jullie ons huis heel? **Opa's en oma,** altijd meelevend en trots op wat ik doe. Ik ben heel erg blij dat jullie deze dag samen met mij kunnen meemaken. Bedankt voor alles. **Marina en Geert Jan**, bedankt voor alle gezelligheid en steun die jullie ons geven!

Lieve **Kim**, samen hebben we al ontzettend veel meegemaakt. Ik ben ontzettend trots op je en de manier waarop jij de afgelopen jaren met alles bent omgegaan. Jij bent heel belangrijk in mijn leven en ik ben blij dat ik alle mooie dingen samen met jou mag delen. We hebben al veel van de wereld gezien, maar ons grote avontuur gaat binnenkort beginnen. Lieve Kim, bedankt dat je me altijd steunt en dat je er altijd voor me bent!

LIST OF PUBLICATIONS

C.C.W.G. Bongers, M. Alsady, T. Nijenhuis, A.D.M. Tulp, T.M.H. Eijsvogels, P.M.T. Deen, M.T.E. Hopman, Acute Impact of Acute versus Prolonged Exercise and Dehydration on Kidney Function and Injury, *Physiological reports, 2018.*

C.C.W.G. Bongers, T.M.H. Eijsvogels, Editorial paper - Time-motion analysis in the big data era: A promising method to assess the effects of heat stress on physical performance Temperature, *Temperature*, 2018.

F. Wardenaar, D. Hoogervorst, J. Versteegen, N. van der Burg, K. Lambrechtse, **C.C.W.G. Bongers**, Real-Time Observations Of Food And Fluid Timing During A 120 km Ultramarathon, *Frontiers in Nutrition*, 2018.

R. Terink, **C.C.W.G. Bongers**, R.F. Witkamp, M. Mensink, J.M.T. Klein Gunnewiek, and M.T.E. Hopman, Effect of Repetitive Long Distance Walking on Plasma Cytokine Profiles, *Scandinavian Journal of Medicine and Science in Sports*, 2018.

C.P. Bogerd, K.B. Velt, S. Annaheim, **C.C.W.G Bongers**, T.M.H. Eijsvogels, H.A.M. Daanen, Comparison of Two Telemetric Intestinal Temperature Devices with Rectal Temperature During Exercise, *Physiological Measurement*, 2018.

C.C.W.G. Bongers, M.T.E. Hopman, T.M.H. Eijsvogels, Comparison of the Validity, Reliability and Inertia Characteristics of Four Different Ingestible Telemetric Temperature Capsule Systems, *Medicine and Science in Sports and Exercise*, 2018.

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C.C.W.G. Bongers, A. Riordan, M.T.E. Hopman, T.M.H. Eijsvogels, T. van Leeuwen, Nieuw licht op de vochtbalans tijdens inspanning, *Sportgericht*, 2016.

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C.C.W.G. Bongers, M.T.E. Hopman, T.M.H. Eijsvogels. Using an ingestible telemetric temperature pill to assess core body temperature, *Journal of Visualized Experiments*, 2015.

C.C.W.G. Bongers, T.M.H. Eijsvogels, J. Nyakayiru, M.T.W. Veltmeijer, D.H.J. Thijssen, M.T.E. Hopman. Thermoregulation and fluid balance during prolonged exercise in 60 versus 80-year old subjects, *Age*, 2014.

C.C.W.G. Bongers, T.M.H. Eijsvogels, M.T.W. Veltmeijer, D.H.J. Thijssen, M.T.E. Hopman. Precooling and Percooling (cooling during exercise) both improve performance in the heat: A Meta-Analytical Review. *British Journal of Sports Medicine*, 2014.

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R.J.H.M. Verheggen, T.M.H. Eijsvogels, M. Catoire, R. Terink, R. Ramakers, **C.C.W.G. Bongers**, M. Mensink, A.R.M.M Hermus, D.H.J. Thijssen, M.T.E. Hopman, Cytokine responses to repeated exercise: delayed adaptation in overweight individuals, *Journal of Science and Medicine in Sport*, 2018. *(submitted)*

M. van Delden, **C.C.W.G. Bongers**, D. Broekens, H.A.M. Daanen, T.M.H. Eijsvogels, Thermoregulatory burden in elite sailing athletes during exercise in the heat: a cross-over study, *International Journal of Sports Physiology and Performance*, 2018. *(submitted)*

R. Terink, D.S.M. ten Haaf, **C.C.W.G. Bongers**, M. Balvers, R.F. Witkamp, M. Mensink, T.M.H. Eijsvogels, J.M.T. Klein Gunnewiek, and M.T.E. Hopman, Changes in iron metabolism during prolonged repeated walking exercise in middle aged men and women, *European Journal of Applied Physiology*, 2018. *(submitted)*

CURRICULUM VITAE

Coen Bongers werd op 21 juli 1990 geboren te Zeeland. In 2008 behaalde hij zijn atheneum diploma aan het Udens College te Uden, waarna hij begon met de opleiding Biomedische Wetenschappen aan de Radboud Universiteit Nijmegen. Daar behaalde hij in 2011 zijn Bachelor diploma om vervolgens de Master Biomedical Sciences, met als specialisatie Human Movement Sciences, in 2013 succesvol af te ronden. Tijdens zijn eerste onderzoeksstage kwam hij voor het eerst in aanraking met de afdeling Integratieve Fysiologie. Onder begeleiding van Dr. Thijs Eijsvogels bekeek hij de effecten van het dragen van een koelvest tijdens inspanning op de sportprestatie en thermoregulatie van atleten, wat resulteerde in zijn eerste publicatie. Gedurende deze stage is zijn interesse in de humane thermoregulatie ontstaan, wat uiteindelijk de basis is geweest voor zijn verdere promotie onderzoek. In oktober 2013 is Coen gestart met zijn promotietraject, waarbij de focus lag op de thermoregulatie en vochtbalans tijdens inspanning. Gedurende zijn promotie heeft hij verschillende studies uitgevoerd naar het effect van koel interventies op de sportprestatie en thermoregulatie. Daarnaast heeft hij een nieuwe methode om de lichaamstemperatuur te meten, middels een temperatuur pil, gevalideerd. Tijdens zijn periode als promovendus heeft hij meerdere bachelor- en masterstudenten begeleid en was hij betrokken bij het onderwijs van Geneeskunde en Biomedische Wetenschappen aan de Radboud Universiteit Nijmegen. Daarnaast is Coen medeorganisator geweest van de Dag van het Sportonderzoek en was hij als hoofdonderzoeker verantwoordelijk voor het jaarlijkse Vierdaagse Onderzoek. Momenteel is Coen als postdoc onderzoeker verbonden aan de afdeling Fysiologie, waar hij verder onderzoek gaat doen naar de thermoregulatie tijdens inspanning om sporters optimaal voor te bereiden op inspanning in de hitte. Tevens gaat Coen voor 6 maanden onderzoek doen aan de Universiteit van Sydney, waar hij in het lab van dr. Ollie Jay een methode gaat ontwikkelen om de zweet efficiëntie op individueel niveau te bepalen.

RIHS PHD PORTFOLIO

Institute for Health Sciences
Radboudumc

PhD candidate: Coen C.W.G. Bongers
Department: Physiology
Graduate School: Radboud Institute for Health Sciences

PhD period: 01-10-2013 – 01-12-2017
Promotor: Prof. Maria T.E. Hopman
Co-promotor: Dr. Thijs M.H. Eijsvogels

TRAINING ACTIVITIES

	Year(s)	ECTS
Courses & Workshops		
RIHS introduction course	2014	1.5
Basiscursus klinisch onderzoekers (BROK)	2014	1.5
Scientific Integrity	2014	1.0
Wetenschapsjournalistiek	2015	3.0
PhD Organisation Nijmegen, course InDesign	2016	0.5
Seminars & lectures		
Prof. Paul Thompson, Can (too much) exercise hurt your heart	2015	0.1
Dr. Thomas Kantermann, How light-styles shape sleep-style	2015	0.1
Prof. Samuel Marcora, The psychobiology of perceived effort during physical tasks	2017	0.1
Prof. James Skinner, The influence of genetic factors on training and health	2017	0.1
Symposia & congresses (OP/PP indicates oral or poster presentation)		
ThermoNed symposium, Amsterdam (OP)	2013	0.5
ThermoNed symposium, Maastricht (OP)	2013	0.5
Rehabilitation conference, Groningen (OP)	2014	1.0
European College of Sport Sciences (ECSS), Amsterdam (OP)	2014	1.5
American College of Sports Medicine, Boston (PP)	2016	1.25
Society for Sports Medicine: Thermophysiology, Bilthoven (OP)	2016	0.5
Scientific meeting Eat2Move, Wageningen (OP)	2016	0.5
American College of Sports Medicine, Denver (PP)	2017	1.25
Annual meeting SevenHills running team, Groesbeek (OP)	2017	0.5
ThermoNed Symposium, Nijmegen and Amsterdam (2x)	2015-2017	0.75

TEACHING ACTIVITIES

	Year(s)	ECTS
Lecturing (Pr = practica, LE= lecture, GA= group assignments, IL= interactive lectures, SSA= self-study assignment)		
BSc course: DT03 – Determinanten 3: Fysische factoren (PR)	2014-2016	1.0
BSc course: 5O101 – Circulatie and Respiratie 1 (PR)	2014-2015	0.25
BSc course: 5O103 – Beweging en Sturing (PR, GA)	2014-2016	1.0
BSc course: 5O104 – Regulatie en Integratie (PR)	2014-2015	0.25
MSc course: 5HM03 - Clinical Exercise Physiology (PR, GA)	2014-2016	3.0
Student education at the Department of Physyiology (LE)	2014-2016	0.25
BSc course: MIN05 – Moving Questions (PR, LE GA, IL)	2015-2017	2.0
BSc course: 6MBB – Belasting en belastbaarheid (PR, GA)	2016	0.25
BSc course: Clinical Exercise Physiology (LE, GA, IL)	2017	1.5
MSc course: Applied Exercise Physiology (LE, GA, IL)	2017	2.0
Development of new education material for clinical exercise physiology and applied exercise physiology (LE, SSA, GA)	2017	1.0
Supervision of internships / other		
BSc interships:		
Stefan Auener – Dehydration efficiency of interval vs continuous exercise	2014	1.0
Niek van Helvoort – Impact of active vs inactive lifestyle on kidney function	2015	1.0
Richard de Boer – Kidney responses during exercise-induced dehydration	2015	1.0
Jan Bookelaar – Determination of lactate levels in human body fluids	2015	1.0
Anouk Tulp – Kidney function during exercise and dehydration in active and inactive	2016	1.5
Michelle van Delden – Thermoregulatory burden in elite sailing athletes	2017	1.0
Iris Cuijpers – Local oxygen consumption during eccentric versus concentric exercise	2017	1.0
MSc internships:		
Hai Ngo – Effects of wearing a cooling vest on thermoregulation of SCI patients	2014	1.0
Paul van Herpt – Fluid homeostasis in heart failure patients during prolonged exercise	2015	0.5
Arnoud Okken – Core body temperature in elite African *vs* Caucasian runners	2015	1.0
Adriaan Penson – Lactate levels in blood, saliva, sweat and urine during exercise	2016	1.0
Jasmijn Dibbits – Ex-vivo comparison of four ingestible temperature sensors	2016	1.0
Daan Slagman – Effects of chronic knee discomfort on quadriceps muscle function	2016	1.0
Nitya van Rijt – Effectiveness of collagen peptide supplementation on knee discomfort	2017	1.5
Yannick de Korte – Prediction of thermoregulatory responses in well trained athletes	2017	1.5
Other		
Reviewer Scientific publication (6x)	2014-2017	0.6
Second assessor of internship reports of BSc students (2x)	2015-2016	0.2
Meet your PhD (mentor of 4 first year Biomedical Sciences students)	2016-2017	0.4
Co-organizing a 1-day conference - Dag van het Sportonderzoek	2015	1
Course coordinator – minor Sport and Exercise Sciences	2016-2017	1
TOTAL		**47.85**

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